

For **AQA** Specifications '**A**'
Advanced Subsidiary

Human Biology **Only**



AS Module 3 **Pathogens & Disease**

FELTHAM PRESS

AS Module 3

(Human Biology Only)

Pathogens and Disease

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Pathogens and Disease

1

Bacteria and Viruses as examples of pathogenic microorganisms

Contents

Bacteria

- ▼ The sigmoid growth curve of a bacterial population. The effect of temperature and nutrient availability on the growth of bacterial populations.

Viruses

- ▼ The structure of the human immunodeficiency virus (HIV) and its replication

The association of microorganisms with disease

- ▼ Diseases as a result of pathogenic organisms penetrating any of the body's interfaces with the environment
- ▼ Microorganisms causing disease by damaging the cells of the host and by producing toxins
- ▼ Illustration of these principles by reference to:

Salmonella sp.

Mycobacterium tuberculosis

HIV

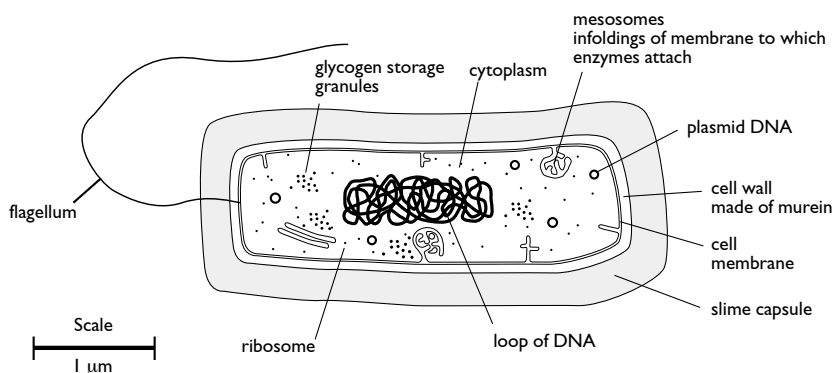
Introduction

Infectious or communicable diseases are caused by a range of organisms including viruses, bacteria, fungi, protoctists, arthropods, platyhelminthes and others which are collectively referred to as **pathogens**. The death toll from such pathogens is staggering: current estimates suggest that in 1999 diarrhoeal diseases caused by a range of bacteria killed almost 3 million people worldwide, HIV/AIDS (caused by a virus) another 2.6 million, tuberculosis (caused by a bacterium) over 1.5 million, and malaria (caused by a protoctist) a similar number. In many developing countries infectious diseases are still the most significant cause of death.

BACTERIA

Of the many thousands of species of bacteria only a relatively small proportion are human pathogens. Some common examples are *Salmonella enteritidis* a cause of food poisoning, *Mycobacterium tuberculosis*, the cause of tuberculosis (TB) and *Streptococcus sp.* a cause of throat infections. Other bacteria live freely in the soil, air or water, or mutualistically in or on animal hosts without causing them harm. Examples are forms of *Escherichia coli* (*E. coli*) which inhabit the large intestine of humans, and *Nitrobacter*, a soil dwelling bacterium which is involved in the nitrogen cycle.

Bacteria



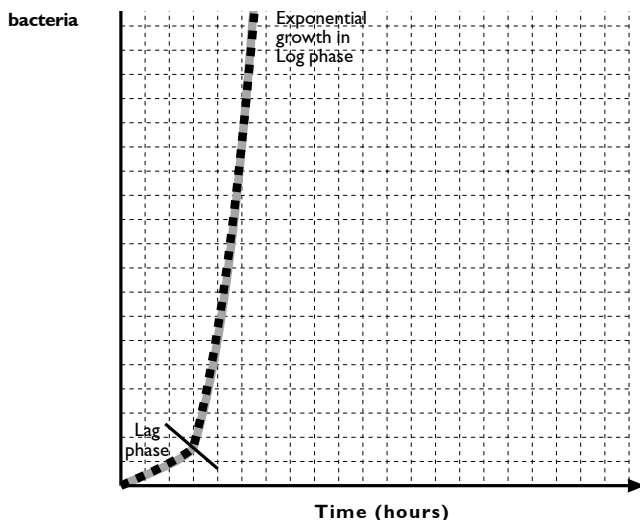
Bacterial Growth

Bacteria reproduce asexually by binary fission (one cell dividing to form two identical daughter cells). The time taken for one cell to become two is highly variable, but it can be around 30 minutes. This doubling of the population every 30 minutes leads to rapid increases in numbers, and can explain why an individual may appear well one day and be severely ill or dead from a bacterial infection the next. The increase in numbers of a bacterial population is referred to as bacterial growth.

Colonies grown in a closed system, e.g. a petri dish, where there are no additions or removals of materials once growth has started, show a characteristic **sigmoid growth curve** when numbers of bacteria are plotted against time (sigmoid means curved like a capital S). Such curves can be derived from laboratory analysis of bacteria growing in a nutrient medium (a **bacterial culture**) which would require sampling the culture and counting the number of bacteria in a sample of equal size at regular time intervals. The curve has four main phases:

- ▼ **Lag phase.** In this initial phase the bacterial culture is getting started, but division is not occurring at a maximum rate. The bacteria are adapting to their new surroundings and possibly synthesising new enzymes to digest the nutrient medium.
- ▼ **Log phase.** This is the period of maximum growth. The population increases by doubling at intervals (e.g. 2->4->8->16->32->64 etc.). If the actual numbers of bacteria are plotted on a graph this phase would be represented by an increasingly steep curve. Usually, however the numbers are converted to logarithms to the base 2. In other words the power to which two must be raised to give that number, e.g. $4 = 2 \times 2 = 2^2$ and the log is 2; $8 = 2 \times 2 \times 2 = 2^3$ and the log is 3, etc. In mathematical terms, 3 is the log of 8 to the base 2. When the logs are plotted they produce a straight line. Thus instead of 2->4->8->16->32->64 etc. the figures would be 1->2->3->4->5->6 etc.
- ▼ **Stationary phase.** After a brief period of growth at a reduced rate, bacterial growth ceases and the numbers stay constant. Some bacteria are still dividing at this point, but an increased death rate prevents any overall (net) increase in numbers. Reasons for this stationary period include depletion of nutrients, depletion of oxygen, and accumulation of waste products.
- ▼ **Decline phase.** In this final part of the curve bacteria stop dividing and the death rate increases. Numbers may eventually fall to zero.

Bacterial Growth Curve



This curve is produced in laboratory investigations of bacteria. In natural habitats such curves are unlikely to be found, as ideal conditions for exponential growth, and a habitat free of competitors or predators is rarely found. When microorganism are grown commercially, for example during the production of insulin in genetically engineered yeast or bacterial cells, then conditions are carefully monitored to ensure optimum growth.

◆ CHECKPOINT SUMMARY

- ◆ Bacteria reproduce asexually by binary fission (one cell dividing by mitosis to form two identical daughter cells). The increase in numbers of a bacterial population is referred to as bacterial growth.
- ◆ The nature of this growth process is such that a characteristic sigmoid growth curve can be plotted with numbers of bacteria against time.
- ◆ The curve has four main phases:
- ◆ Lag phase in which the bacterial culture is getting started, but replication is not occurring at a maximum rate.
- ◆ Log phase which is the period of maximum growth. The population increases by doubling at intervals (e.g. 2->4->8->16->32->64 etc.).
- ◆ Stationary phase. After a brief period of growth at a reduced rate, bacterial growth ceases and the numbers stay constant.
- ◆ Decline phase. In this final part of the curve bacteria stop dividing and the death rate increases. Numbers may eventually fall to zero.
- ◆ In natural habitats such curves are unlikely to be found as ideal conditions for exponential growth and a habitat free of competitors or predators is rarely found.

Bacteria culture at 30°C

Number of cells in millions		
Time/h	Living	Living & Dead
0	9	10
1	10	11
2	11	12
5	18	20
10	400	450
12	550	620
15	550	700
20	550	850
30	550	950
35	225	950
45	30	950

VIRUSES

Viruses are structures from 20-300 nm in size, which contain genetic material in the form of DNA **or** RNA (never both) and infect cells of other organisms (prokaryotic and eukaryotic). There is much debate as to whether they should be defined as living organisms since although they possess genetic material, they lack many of the basic features shown by all other living organisms, including the presence of a cellular structure. Viruses are incapable of reproducing themselves outside the environment of a host cell.

Whilst details vary, all viruses have similar structural features which include a central core of genetic material (DNA or RNA never both) and a capsid or protective protein coat around the core formed of units called capsomeres.

As a group, viruses cause an enormous amount of damage and death amongst organisms of all five kingdoms (bacteria, fungi, protoctista, plants and animals) yet each virus is very specific to one particular type of cell in one host species. In humans, viral diseases include chicken pox, measles, influenza, and HIV/AIDS.

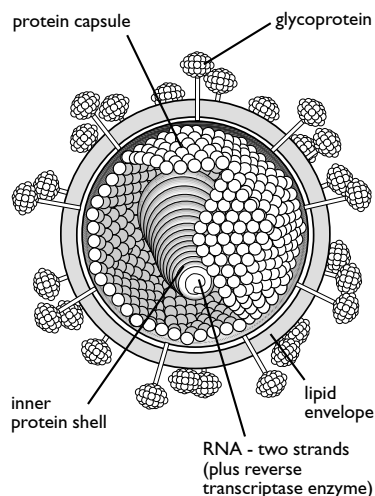
Human Immunodeficiency Virus (HIV)

HIV is a virus which causes the disease known as AIDS (Acquired Immune Deficiency Syndrome). This disease is one of the fastest growing causes of death in the world, and is currently responsible for about 3 million deaths per year. HIV infects a type of white cell known as the T-helper lymphocyte or CD4 cell. In doing so it severely damages the immune system of infected people, making them highly susceptible to infection and death from a range of infectious diseases and cancers.

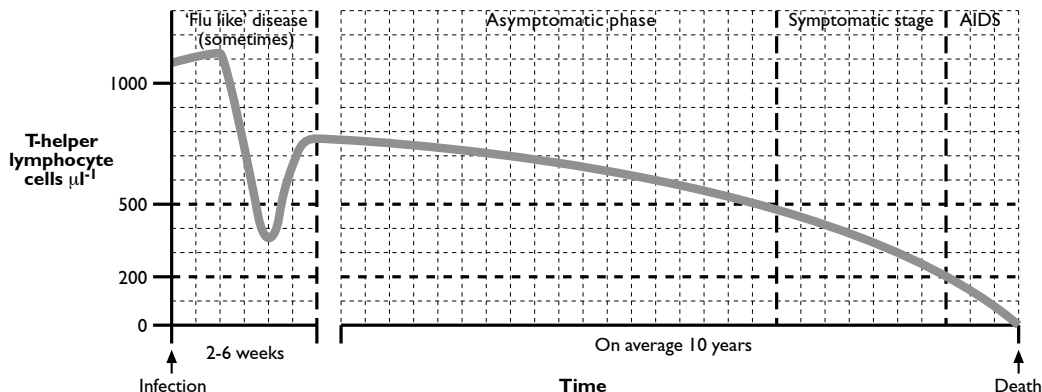
Structure of HIV

HIV belongs to a group of viruses known as **retroviruses**. The main feature of such viruses is the presence of RNA rather than DNA as the genetic material in the core of the virus. The HIV virus has two identical RNA strands. Also present is the enzyme reverse transcriptase, which allows single stranded RNA to be converted to double stranded DNA. The RNA is encapsulated in layers of proteins, glycoproteins and lipids.

Human Immunodeficiency Virus



Decline of T-helper lymphocytes in the course of an HIV infection



Replication of HIV

Like all viruses, HIV is incapable of independent replication. It relies on inserting its genetic material into the DNA of human T-helper cells and allowing these cells to manufacture new copies of the viral RNA and proteins necessary for assembling new viruses. There are several stages in this process:

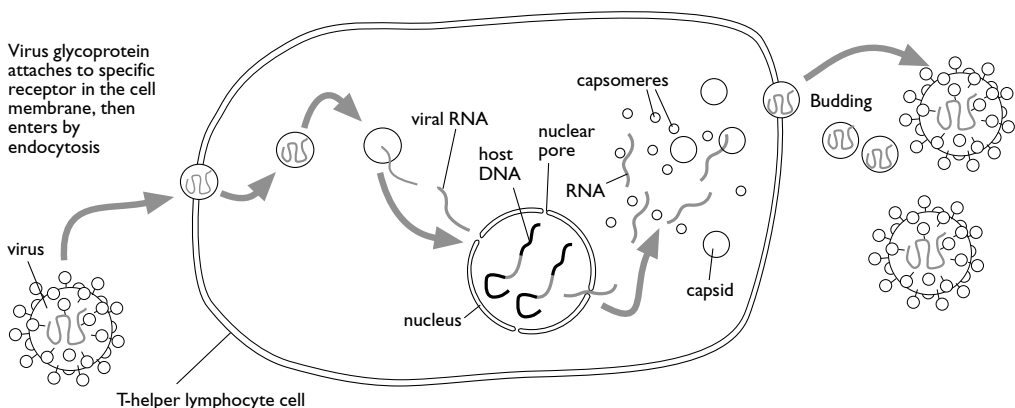
- ▼ HIV enters the body from an infected (HIV positive) person via body fluids such as blood or semen.
- ▼ It attaches to specific receptors on the cell membranes of T-helper lymphocytes.
- ▼ HIV enters the cell by endocytosis releasing its RNA and reverse transcriptase enzymes into the cytoplasm.
- ▼ The RNA strand is copied to form a double stranded DNA molecule using reverse transcriptase.
- ▼ The DNA copy inserts itself into the human DNA in the nucleus of the T-helper lymphocyte.
- ▼ After a variable period of time (latency period) the DNA is copied (transcribed) to make new viral RNA. At the same time, the viral proteins necessary for the capsid and envelope proteins are synthesised by the cell.
- ▼ New viruses are assembled from the RNA and proteins and leave the cell by exocytosis. During this process the viral envelope is constructed from the cell membrane of the host cell.

As with any virus, large numbers of new HIV viruses are made by a single cell, all of which may, in turn, infect new cells. Since HIV eventually kills the T-helper lymphocytes it has infected, one of the signs that an HIV infection has become active is a rapid decrease in the numbers of these cells. T-helper lymphocyte counts are used in the monitoring of HIV infection. Once the levels drop below 200 cells per μl of blood AIDS is diagnosed.

◆ CHECKPOINT SUMMARY

- ◆ Viruses are structures from 20-300nm in size, which contain genetic material in the form of DNA or RNA (never both) and infect cells of other organisms (prokaryotic and eukaryotic).
- ◆ Viruses are incapable of reproducing themselves outside the environment of a host cell.
- ◆ All viruses have similar structural features which include a central core of genetic material (DNA or RNA) and a capsid or protective protein coat around the core formed of units called capsomeres.
- ◆ HIV is a virus which causes the disease known as AIDS (Acquired Immune Deficiency Syndrome).
- ◆ HIV infects a type of white blood cell known as the T-helper lymphocyte or CD4 cell. In doing so it severely damages the immune system of infected people, making them highly susceptible to infection and death from a range of infectious diseases and cancers.
- ◆ HIV belongs to a group of viruses known as retroviruses. The main feature of such viruses is the presence of RNA rather than DNA as the genetic material in the core of the virus. The RNA is encapsulated in layers of proteins, glycoproteins and lipids.
- ◆ Like all viruses, HIV is incapable of independent replication. It relies on inserting its genetic material into the DNA of human T-helper cells and allowing these cells to manufacture new copies of the viral RNA and proteins necessary for assembling new viruses.

Infection of a T-helper lymphocyte with HIV



The ASSOCIATION of MICROORGANISM with DISEASE

Koch's postulates

Robert Koch, a nineteenth century bacteriologist was one of the first people to make a definite link between bacteria and specific infectious diseases. He is best known for his work in identifying the bacterium responsible for tuberculosis (*Mycobacterium tuberculosis*). Before Koch, microscopes were not powerful enough to allow bacteria to be seen, and thus many diseases which we now know to be caused by microorganisms were then said to be due to climatic, dietary or other causes.

Koch established three postulates (criteria) which must be fulfilled if an infective cause for a disease (such as bacteria or virus) is to be proven.

- ▼ The organism must be sufficiently abundant in every case to account for the disease
- ▼ The organism associated with the disease can be cultured (grown in the laboratory) artificially in pure culture
- ▼ The cultivated organism produces the disease upon inoculation into another member of the same species.

These are still as relevant today as in Koch's era although a few points should be noted. Firstly, some organisms are sparse and difficult to find in the body even though they are the definite cause of infection. Secondly, some organisms are difficult to culture in the laboratory, even though they have been proven to cause the disease. And thirdly it is unethical to deliberately infect humans with microorganisms so experimental animals must be used instead.

When considering Koch's postulates it is worth remembering that new diseases are still appearing whose cause needs to be discovered. HIV/AIDS, which first appeared in a significant number of cases in San Francisco in the early 1980s, is a good example of a disease whose cause was unknown for some time.

Entry of microorganism into the body

Disease causing microorganisms (pathogens) from the environment can enter the body in several different ways. Some pathogens have found complex modes of entry although most are opportunistic and enter when the body's basic defences against pathogens are breached.

The main points of entry are as follows:

- ▼ Through the skin when it is cut or damaged and thus ceases to act as a barrier to infection. In this case microorganism may enter the blood at the site of the wound. For example tetanus occurs when the bacterium *Clostridium tetani* enters a wound. HIV is most commonly transmitted during sexual intercourse when infected semen from one partner enters the blood of another via a cut or tear in the epithelium of the vagina or rectum.
- ▼ Through the mucous membranes of the respiratory tract when air containing droplets of infectious material are breathed in. This is the way in which *Mycobacterium tuberculosis* the bacterium which causes TB is transmitted. Cold and influenza viruses are also transmitted in this way.

▼ Through the digestive tract when food or water containing microorganisms is taken in. The bacterium responsible for cholera (*Vibrio cholerae*) enters via drinking water infected with faeces whilst *Salmonella enteritidis*, responsible for food poisoning enters when undercooked contaminated food, e.g. chicken meat or eggs are eaten. Microorganisms which cause infection via this route must be resistant to the acidic conditions of the stomach which offer protection against other microorganisms.

Other more complex means of entry of microorganisms into the body include transmission by insect vectors (such as malaria where the *Plasmodium* parasite is injected into the blood when the mosquito vector takes a blood meal) and direct entry through the intact skin (as in Schistosomiasis where the larval stage schistosome burrows in though the skin of the feet.) Both of these organisms are discussed in section 12.2.

How microorganisms cause disease

Once inside the body, microorganisms cause disease in a variety of different ways. In some cases the microorganisms actually damage and destroy the host cells. In other cases the microorganisms themselves do not directly cause harm, but produce toxins which are harmful. In still others it is the body's own immune response to the presence of the microorganism which produces the symptoms. These categories are not, however, mutually exclusive: some microorganisms will cause illness in all three ways.

Microorganism also vary considerably in the numbers of organisms required to cause illness: thus whilst very large numbers of *Salmonella enteritidis* bacteria are required to produce the symptoms of common 'food poisoning', only very small numbers of *Salmonella typhi* are required to cause the more serious symptoms of typhoid.

HIV infection is an example of a pathogen causing direct damage to human cells which in turn affect critical functions within the body. As discussed above, HIV only infects one type of cell: the T-helper lymphocyte, cells which play a vital role in the human immune system. Damage to these cells occurs during viral replication. As the infection progresses, more and more T-helper lymphocytes are infected and destroyed resulting in the condition of **Acquired Immune Deficiency Syndrome** (AIDS). Here the patient is unable to mount immune responses against common pathogens since the T-helper lymphocytes 'help' B and T lymphocytes to work.

Salmonella bacteria also cause direct damage to cells. When these bacteria arrive in the intestine they are taken up by epithelial cells. This process destroys the brush border of the microvilli, creating a characteristic 'ruffled' cell surface. These invaded cells then detach from the intestine wall, creating inflamed lesions. The effect of this is to cause the secretion of large amounts of watery fluid into the lumen of the gut, resulting in the characteristic diarrhoea experienced during an infection with *Salmonella*. It is interesting to note that species of *Salmonella* which do not cause illness are incapable of invading the intestinal lining.

Whilst mucosal damage may explain some of the features of *Salmonella* infections, some scientists believe that toxins produced by the bacteria are significant in causing disease. Many *Salmonella* species have been shown to produce toxins in vitro (in the laboratory)

but their role in the disease is not yet clear. The fact that such bacteria can cause illness within 6 hours of infection suggests to some that a preformed toxin (already present inside bacterial cells when they infect a person) is involved.

Clearer examples of the role of toxins in causing disease are illustrated by the cholera causing bacterium *Vibrio cholerae*, and members of the *E. coli* group of bacteria. *V. cholerae* bacteria themselves are relatively harmless but produce two harmful 'exotoxins' (a toxin released from the cell). One of these toxins causes a loss of chloride and hydrogencarbonate ions from the intestinal cells followed by an osmotic loss of up to 10 litres of water per day. Absorption of water and salts from the gut is also impaired. Together these facts explain the severe watery diarrhoea and death from dehydration which is characteristic of the disease.

Mycobacterium tuberculosis falls into the third basic category described above: that is the effect of the bacterium on the lungs (and indeed other parts of the body) is a result of an immune response by the body to the bacterium. In this case the body tries to destroy the invading bacteria, by walling them off in 'tubercles'. In doing so, however, inflammation and damage to the surrounding cells occurs. These damaged regions (lesions) may become hard or spongy, or liquefy leaving 'holes' in the lungs, sometimes damaging blood vessels. If many such lesions occur lung function is drastically reduced.

◆ CHECKPOINT SUMMARY

- ◆ Koch established three postulates (criteria) which must be fulfilled if an infective cause for a disease (such as a bacteria or virus) is to be proven.
- ◆ The organism must be sufficiently abundant in every case to account for the disease
- ◆ The organism associated with the disease can be cultured (grown in the laboratory) artificially in pure culture
- ◆ The cultivated organism produces the disease upon inoculation into another member of the same species.
- ◆ Disease causing microorganisms (pathogens) can enter the body through the skin when it is cut or damaged. HIV is most commonly transmitted during sexual intercourse when infected semen from one partner enters the blood of another via a cut or tear in the epithelium of the vagina or rectum.
- ◆ Through the mucous membranes of the respiratory tract when air containing droplets of infectious material are breathed in. This is the way in which *Mycobacterium tuberculosis* the bacterium which causes TB, and cold and influenza viruses, are transmitted.
- ◆ Through the digestive tract when food or water containing microorganisms is taken in. *Salmonella enteritidis*, responsible for food poisoning enters when undercooked contaminated food is eaten.
- ◆ Microorganisms cause disease in a variety of different ways. In some cases the microorganisms actually damage and destroy the host cells. In other cases the microorganisms themselves do not directly cause harm, but produce toxins which are harmful. In still others it is the body's own immune response to the presence of the micro-organism which produces the symptoms.
- ◆ HIV infection is an example of a pathogen causing direct damage to human cells which in turn affect critical functions within the body. As discussed above, HIV only infects one type of cell: the T-helper lymphocyte, cells which play a vital role in the human immune system.
- ◆ As the infection progresses more and more T-helper lymphocytes are infected and destroyed resulting in the condition of Acquired Immune Deficiency Syndrome (AIDS). Here the patient is unable to mount immune responses against common pathogens since the T-helper lymphocytes 'help' B and T lymphocytes to work.
- ◆ *Salmonella* bacteria also cause direct damage to cells. When these bacteria arrive in the intestine they are taken up by epithelial cells. These invaded cells then detach from the intestine wall, creating inflamed lesions. The effect of this is to cause the secretion of large amounts of watery fluid into the lumen of the gut, resulting in diarrhoea
- ◆ Toxins produced by bacteria may be significant in causing disease e.g. cholera
- ◆ *Mycobacterium tuberculosis* falls into the third basic category described above: that is the effect of the bacterium on the lungs (and indeed other parts of the body) is a result of an immune response by the body to the bacterium.

2.

Parasites and parasitism

Contents

- ▼ The principal adaptations of parasites to their way of life as illustrated by *Plasmodium* and *Schistosoma*
- ▼ These organisms studied in sufficient detail to illustrate:
 - their ability to survive in the hostile environment within the host
 - reduction of locomotory and other structures
 - modification of reproduction and the life cycle associated with infecting a new host

Introduction

A parasite is an organism which lives on or in a host, causing harm to the host whilst gaining benefits from it. These benefits include an unlimited supply of readily available nutrients and water, and constant optimum temperature. There are many different types of parasites of humans, which include bacteria, protoctists, viruses, fungi, arthropods, and platyhelminthes. Parasites are responsible for a large proportion of all human deaths in many developing countries, as well as having a very significant impact on domestic animals and plant crops.

Parasitic organisms often show a range of unique structural and functional adaptations to their lifestyle which distinguish them from non-parasitic relatives. Some key features include:

- ▼ Specialised reproductive strategies aimed at overcoming the unpredictability of finding new hosts, including producing vast quantities of offspring during phases of asexual reproduction.
- ▼ Modification of mouthparts, digestive systems and enzymes to allow attachment to the host and utilisation of host's food supply, blood or tissues.
- ▼ Features to resist attack by host enzymes or immune system. This may include having a tough protective cuticle to resist attack by host enzymes as well as strategies to evade the immune system.
- ▼ Reduction of unnecessary sensory organs and locomotory organs in the adult stages (especially in larger parasites) as they live in protected, optimum conditions surrounded by readily available nutrients and water.

The malarial parasite, *Plasmodium*.

Plasmodium is a single celled intracellular parasite of the Kingdom Protoctista (Class Protozoa) which causes the disease malaria in humans. It infects approximately one third of the world's population and is responsible for over 1 million deaths per year. It has a complex life cycle which involves two hosts: the human and the female mosquito (*Anopheles sp.*), both of which are essential for its development. Whilst the human host is often severely affected by the parasite, the female mosquito host seems unaffected. Inside the human *Plasmodium* feeds on haemoglobin inside the red blood cells.

The life cycle of *Plasmodium* includes an asexual reproductive phase in the human (in liver cells and then red blood cells) and a sexual reproductive phase in the female mosquito. In spite of the complexity of the cycle and the relatively long time needed for its completion (2-3 weeks) the parasite is very successful in being transferred from one host to another, mainly as a result of the asexual phase producing enormous quantities of parasites (merozoite stage), and the sexual phase generating the genetic diversity which is essential for evading the host's immune system.

The parasite is transferred from human to female mosquito and female mosquito to human via the 'bite' of the female mosquito. When the female mosquito pierces the skin to take blood from a human, it secretes anti-coagulant saliva to prevent clotting of the blood. If the mosquito is infected with the malarial parasite, malarial parasites in the **sporozoite** form are injected into the human.

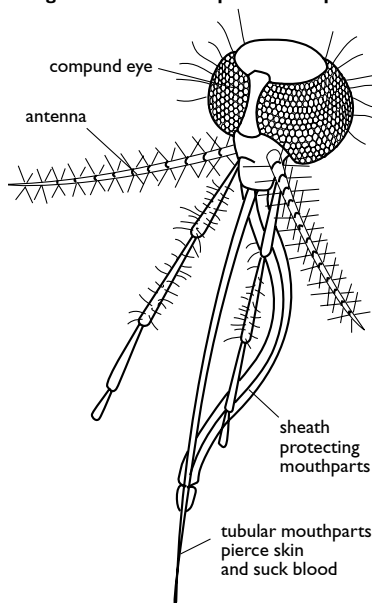
If the human is infected with the malarial parasite, the female mosquito takes up *Plasmodium* gametes in the blood on which the mosquito feeds.

Inside the human body *Plasmodium* is able to evade the immune system. Initially it lives inside the liver cells and subsequently inside the red blood cells, and cannot be targeted by the hosts immune system.

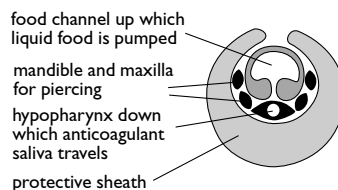
Life cycle

A malaria infection of a human starts when a female mosquito injects anticoagulant saliva containing young malarial parasites known as sporozoites into the blood of a human on which it is feeding. These parasites migrate to the liver cells, undergo asexual reproduction, develop into **merozoites** and then infect red blood cells. Here they also reproduce asexually by multiple fission producing huge numbers of additional merozoites which burst out of the red blood cells and cause the characteristic fever and other disease symptoms. While most merozoites enter new red blood cells for further rounds of asexual reproduction, some develop into **gametocytes** which can be taken up when another female mosquito feeds on the infected person's blood. Once inside the body of the female mosquito the gametes produced by these gametocytes fuse, and the resulting zygotes develop into sporozoites which move into the salivary glands of the female mosquito where they can be injected into another person. The cycle thus begins again.

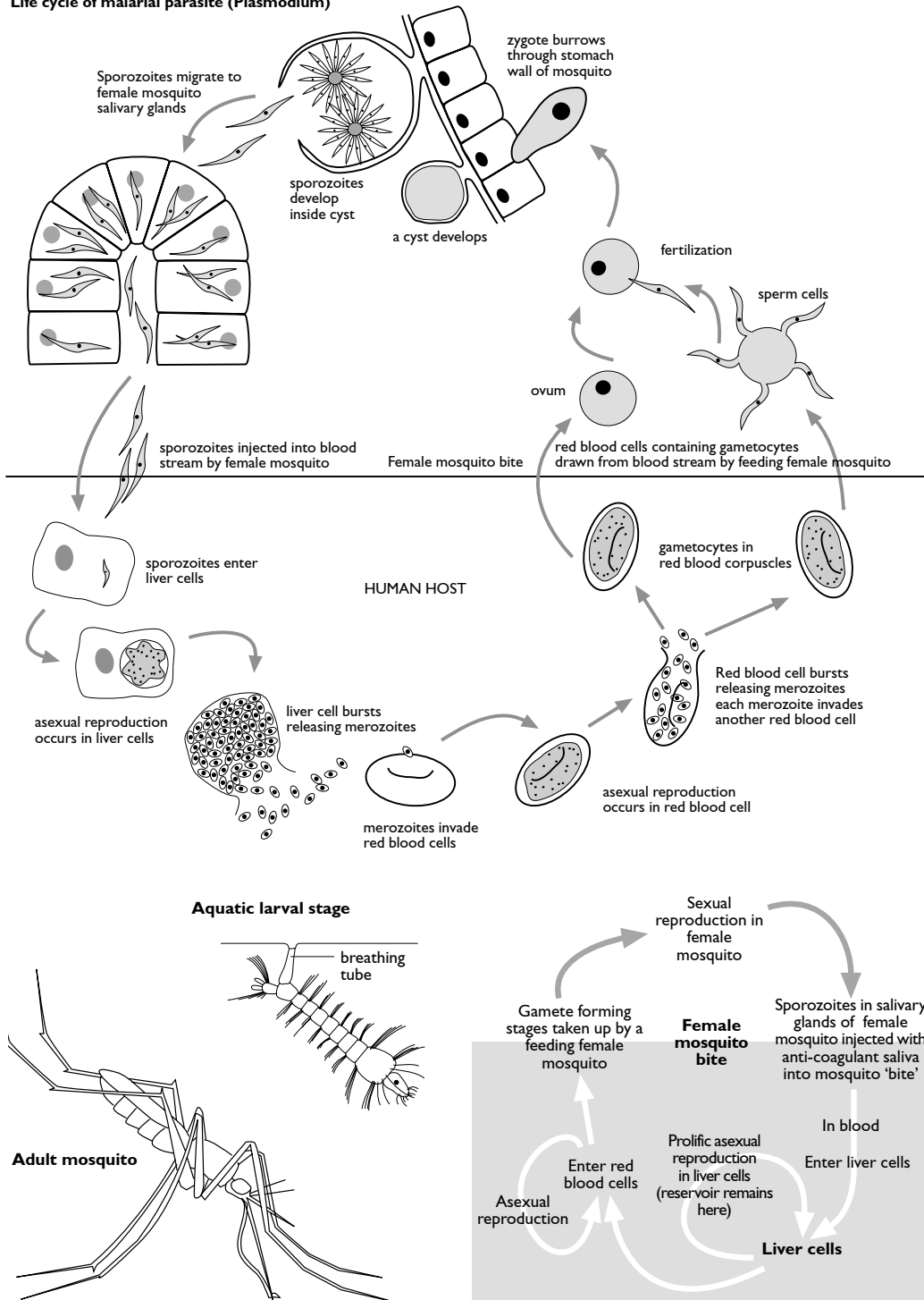
Diagram to show mosquito mouthparts



Section through mouthparts



Life cycle of malarial parasite (*Plasmodium*)



Schistosoma

Schistosoma is the name of a genus of parasitic flatworms (Phylum Platyhelminthes: Class Trematoda) which cause the disease schistosomiasis (formerly known as Bilharzia) in humans. This disease is thought to affect over 200 million people across the tropics. *Schistosoma* is an endoparasite, inhabiting the blood vessels of the bladder region (*Schistosoma haematobium*) or of the intestine (*S. mansoni* and *S. japonicum*). It attaches to the vessels via suckers and feeds directly on blood which it digests using a protease enzyme in its gut.

Like *Plasmodium*, *Schistosoma* has a complex life cycle involving two hosts. In this case certain fresh water snails are the non-human hosts. The complexity of the life cycle and relatively small chance of finding a new host is overcome by the enormous numbers of larvae produced during asexual reproduction.

Life cycle

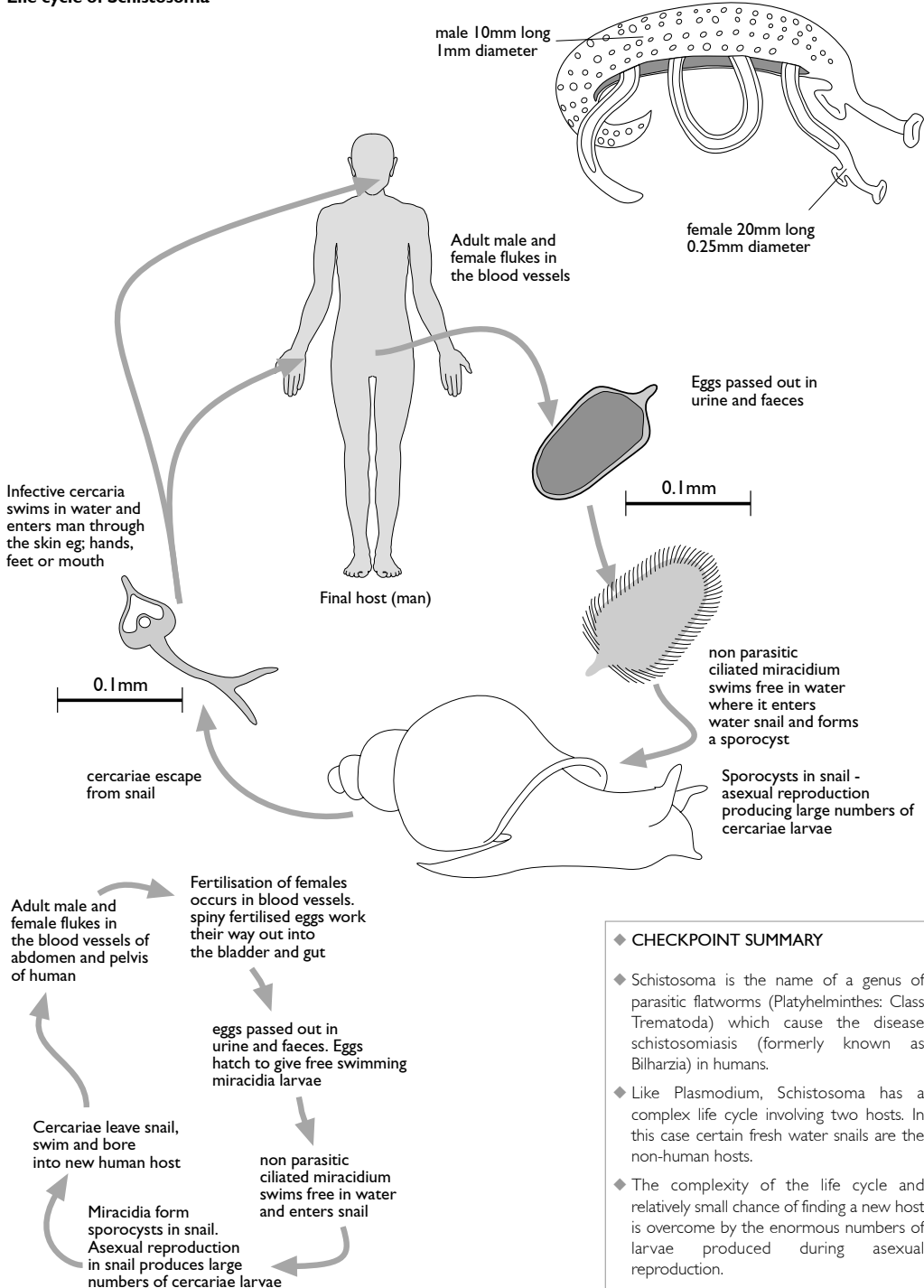
The life cycle of *Schistosoma* relies on water, and on the contamination of water by human urine and faeces. During the life cycle eggs pass out of an infected human via urine or faeces. They hatch into minute ciliated larvae (**miracidia**) which are capable of swimming until they find and burrow into the flesh of a water snail. Asexual reproduction occurs here producing free swimming larvae (**cercariae**) which burrow (aided by the secretion of digestive enzymes) into the human via the skin of the feet. The larvae then migrate either to the bladder or gut region where they may exist for many years. The importance of this asexual stage in generating vast numbers of parasites cannot be overemphasised: a single miracidium may give rise to 200,000 cercariae over a period of a few months.

Adult schistosomes exist as separate males and females which, are usually found attached to one another, to ensure mating and sexual reproduction. (A single human may only possess a few adult forms and if they did not attach to one another they might never meet to mate). The adult worms lack any means of locomotion and have no sensory organs as these are unnecessary in their habitat inside the host's body, where they are surrounded by nutrients in constant optimum conditions.

◆ CHECKPOINT SUMMARY

- ◆ A parasite is an organism which lives on or in a host, causing harm to the host whilst gaining nutritional and other benefits from it.
- ◆ Parasitic organisms often show a range of unique structural and functional adaptations to their lifestyle which distinguish them from non-parasitic relatives. Some key features include:
 - ◆ Specialised reproductive strategies aimed at overcoming the unpredictability of finding new hosts including producing vast quantities of offspring during phases of asexual reproduction.
 - ◆ Modification of mouthparts, digestive systems and enzymes to allow attachment to the host and utilisation of host's food supply, blood or tissues.
 - ◆ Features to resist attack by host enzymes or immune system. This may include having a tough protective cuticle to resist attack by host enzymes as well as strategies to evade the immune system.
 - ◆ Reduction of unnecessary sensory organs and locomotory organs in the adult stages (especially in larger parasites).
 - ◆ *Plasmodium* is a single celled intracellular parasite of the Protocist kingdom (Class Protozoa) which causes the disease malaria in humans.
 - ◆ It has a complex life cycle which involves two hosts: the human and the female mosquito (*Anopheles* sp.), both of which are essential for its development. Inside the human *Plasmodium* feeds on haemoglobin inside the red blood cells.
 - ◆ The life cycle of *Plasmodium* includes an asexual reproductive phase in the human (in liver cells and then red blood cells) and a sexual reproductive phase in the female mosquito.
 - ◆ The asexual phase produces enormous quantities of parasites (merozoite stage), and the sexual phase generates the genetic diversity which is essential for evading the hosts immune system.
 - ◆ When the female mosquito takes a blood meal from a human, malarial parasites are injected into the wound with the anti-coagulant saliva from the salivary glands, whilst *Plasmodium* gametes are taken up in the blood on which the mosquito feeds.

Life cycle of *Schistosoma*



◆ CHECKPOINT SUMMARY

- ◆ *Schistosoma* is the name of a genus of parasitic flatworms (Platyhelminthes: Class Trematoda) which cause the disease schistosomiasis (formerly known as Bilharzia) in humans.
- ◆ Like *Plasmodium*, *Schistosoma* has a complex life cycle involving two hosts. In this case certain fresh water snails are the non-human hosts.
- ◆ The complexity of the life cycle and relatively small chance of finding a new host is overcome by the enormous numbers of larvae produced during asexual reproduction.

3**Defensive functions of mammalian blood****Content****General mechanism of defence against disease**

- ▼ Phagocytosis and the subsequent destruction of ingested pathogens
- ▼ The roles of thromboplastin, prothrombin, plasma enzymes, calcium ions and fibrinogen in blood clotting

Principles of immunology

- ▼ Definitions of antigen and antibody
- ▼ The essential difference between humoral and cellular responses as shown by B-lymphocytes and T-lymphocytes. The role of plasma cells and memory cells in producing primary and secondary response

Passive and active immunity

- ▼ Passive immunity
- ▼ Antibodies acquired through the placenta and via lactation and those acquired artificially
- ▼ Vaccination and immunisation
- ▼ Attenuated and dead microorganisms, and genetic engineering as the basis for vaccines

GENERAL MECHANISM of DEFENCE AGAINST DISEASE

In order to remain healthy and able to function properly, the human body must protect itself from disease-causing organisms, known as pathogens. Firstly it must aim to prevent pathogens from entering the body in the first place, and secondly it must be able to defend itself against them if they do enter. The first lines of defence are therefore the barriers to infection, which include the following.

- ▼ The impermeable skin, the surface layers of which are made up of many layers of dead, tough, keratin impregnated cells.
- ▼ The blood clotting process, which is rapidly activated to plug any wound and prevent pathogen entry.
- ▼ The mucous membranes of nose and respiratory tract which trap pathogens in sticky mucus, which is moved away from the delicate lining of the alveoli by cilia.
- ▼ Anti-bacterial enzymes in saliva, sweat and tears.
- ▼ Stomach acid (pH 2) which kills most pathogens entering with the food.

If pathogens do manage to pass through these barriers and enter the body tissues or blood, an immune system is necessary to attack and destroy these pathogens and so prevent serious disease or death. There are two branches of the immune system in operation within the body which co-operate with each other to some extent. These are:

- ▼ **Non-specific immunity** which is not targeted at specific types of pathogen but at foreign material in general. This involves phagocytosis and inflammation and is immediate.
- ▼ **Specific immunity** which uses B and T lymphocytes to target specific pathogens. Such responses are more complex and are often delayed.

Phagocytosis

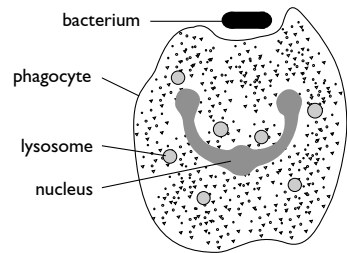
Phagocytosis is the process by which certain white cells (phagocytes) engulf and digest pathogens, other foreign material and dead or infected cells. These phagocytes are non-specific in their actions so will deal with any foreign material which they meet. One type of phagocyte (neutrophil) primarily engulfs bacteria, whilst another (macrophage), being larger, is capable of engulfing larger particles and cells including old or infected red blood cells.

The phagocytes are found in blood, lymph and the tissues, as they are capable of squeezing through the tiny gaps between cells in the walls of capillaries and entering the tissue fluid. This is important as it allows them to move to any body tissue which becomes infected with pathogens.

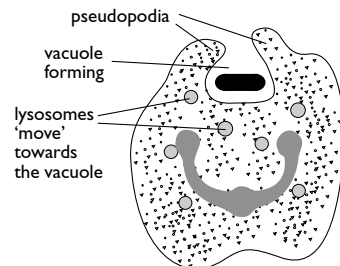
The process of phagocytosis in both neutrophils and macrophages involves several stages.

- ▼ The phagocyte comes into direct contact with the foreign particle or microorganism. In some cases phagocytes are attracted by chemical signals from damaged cells or invading bacteria and move towards them (chemotaxis). Some bacteria may become coated with opsonins (a type of antibody) which allows phagocytes to bind to them.

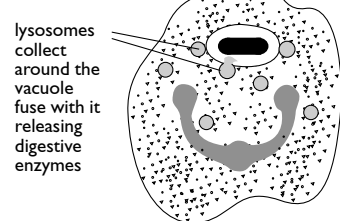
Phagocyte 'moves' towards bacterium



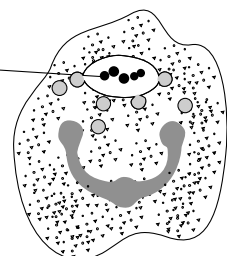
Vacuole forming



Vacuole formed



bacterium digested and products absorbed



- ▼ The phagocyte begins the ingestion process. The cell membrane and cytoplasm move out forming 'pseudopodia' around the particle or microorganism.
- ▼ The particle or microorganism is enclosed in a vacuole formed from the cell membrane of the phagocyte.
- ▼ Lysosomes fuse with the vacuole and their digestive enzymes and other chemicals digest the particle or microorganism, and the products of digestion are absorbed by the phagocyte or released from the cell.

Blood clotting

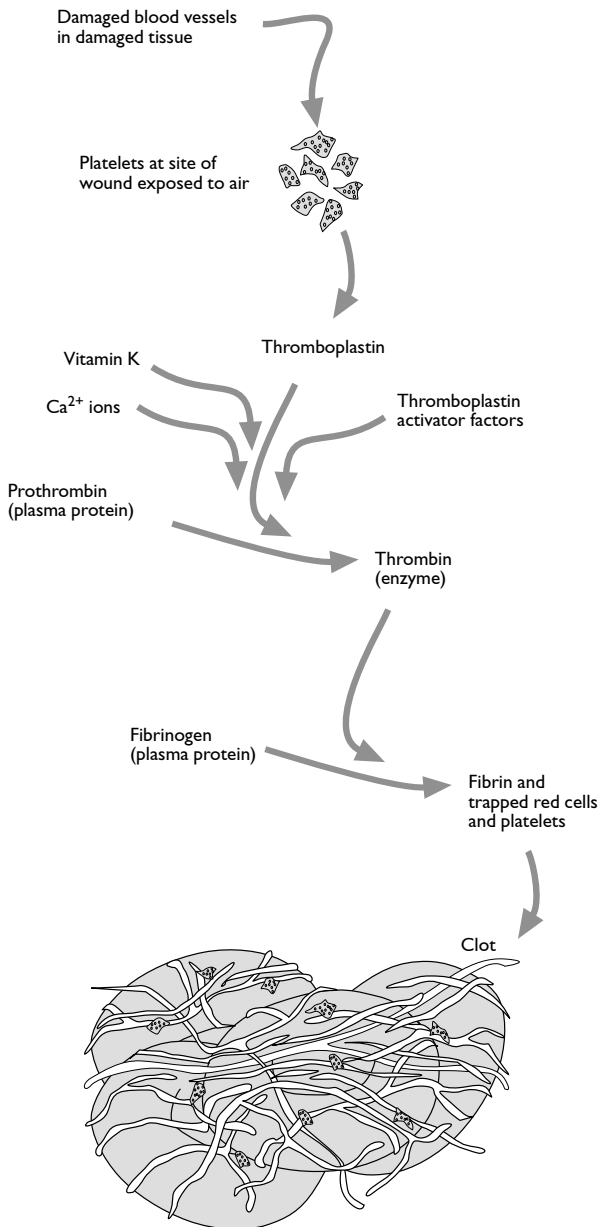
When intact the skin provides an excellent barrier against the entry of microorganisms that cause disease (pathogens). It is waterproof and impermeable to pathogens, whilst sweat and sebum contain anti-bacterial agents to further reduce the risk of infection. If, however, the skin is cut or damaged pathogens may easily gain entry to the tissues and blood and spread through the whole body. At the same time blood may escape from the wound, causing illness or even death. To limit the extent of both blood loss and infection mammals have evolved a blood clotting mechanism which allows the rapid formation of a hard clot or scab at the site of a wound.

The clotting process, which starts within seconds of skin damage, involves a series of stages, (a cascade) catalysed by chemicals known as 'clotting factors'. Many of these chemicals are enzymes and occur in the blood plasma in inactive forms until the clotting process starts: this is important as it prevents blood clotting inside blood vessels under normal conditions. The clotting process can be summarised as follows:

- ▼ Blood escapes from skin wound and comes into contact with air.
- ▼ Platelets and damaged tissues at the wound site release a number of substances including thromboplastin, several clotting factors (including factor VIII) and calcium ions
- ▼ Thromboplastin and other substances catalyse the conversion of inactive prothrombin to thrombin, an active protease enzyme. Calcium ions are necessary for this stage.
- ▼ Thrombin converts the soluble protein fibrinogen in the plasma to its insoluble form fibrin.
- ▼ The insoluble fibrin forms a dense mesh of cross-linked fibres which traps platelets and red blood cells around the wound. Platelets are activated and become sticky, attaching to the fibrin fibres forming a dense plug and also to the walls of any damaged vessels.
- ▼ Gradually the fibrin fibres pull the clot in tighter so that it completely plugs the wound.

Mutations in the genes coding for the plasma clotting factors result in a reduction in the efficiency of the blood clotting process. Collectively these conditions, which are inherited in a sex-linked manner, are known as haemophilia. In one of the most common types a gene responsible for the production of clotting factor VIII is faulty leading to the absence of this factor. In the past such individuals would often die from small wounds or internal bruises where the blood failed to clot. Now genetically engineered factor VIII produced in sheep is available for treatment of haemophiliacs.

Blood clotting



◆ CHECKPOINT SUMMARY

- ◆ If the skin is broken the blood clotting process is rapidly activated to plug the wound and prevent pathogen entry.
- ◆ The mucous membranes of nose and respiratory tract trap pathogens in the sticky mucus, which is moved away from the delicate lining of the alveoli by cilia.
- ◆ Anti-bacterial enzymes are found in saliva, sweat and tears
- ◆ Stomach acid (pH 2) kills pathogens in the food.
- ◆ Non-specific immunity is targeted at foreign material in general. Involves phagocytosis and inflammation and is immediate.
- ◆ Phagocytosis is the process by which pathogens, other foreign material and dead or infected cells are engulfed and digested by phagocytes. These phagocytes are non-specific in their actions.
- ◆ Specific immunity uses B and T lymphocytes to target specific pathogens. Such responses are more complex and are often delayed.
- ◆ Blood clotting involves a series of stages, (a cascade) catalysed by chemicals known as 'clotting factors'.
- ◆ Platelets and damaged tissues at the wound site release a number of substances including thromboplastin, several clotting factors (such as factor VII and factor X) and calcium ions
- ◆ Thromboplastin and other substances catalyse the conversion of prothrombin (inactive) to thrombin, an active protease enzyme. Calcium ions are necessary for this stage.
- ◆ Thrombin converts the soluble protein fibrinogen in the plasma to its insoluble form fibrin by hydrolysis.
- ◆ The insoluble fibrin forms a dense mesh of cross-linked fibres which traps platelets and red blood cells around the wound. Platelets are activated and become sticky, attaching to the fibrin fibres forming a dense plug and also to the walls of any damaged vessels.
- ◆ Gradually the fibrin fibres pull the clot in tighter so that it completely plugs the wound.

Principles of immunology.

Immunology is the study of the mechanisms by which foreign material entering the body is recognised and destroyed thus protecting the individual from harm. Such foreign material may be in the form of pathogens (disease-causing bacteria, fungi, viruses and protoctists), in the form of human cells from other individuals (as in blood transfusions or organ transplants), or, in the case of allergic reactions, in the form of molecules on common products like pollen and peanuts. In all cases, foreign or 'non-self' material is distinguished from 'self' material by the presence of antigens, and responses are specific to the type of antigen encountered.

Antigens

Antigens are defined as molecules, which, when foreign to an individual, stimulate an immune response by lymphocytes in the body, in particular the production of antibodies.

Antigens are often protein or glycoprotein molecules found on the cell surface membrane, but a range of other molecules, including inorganic molecules can also act as antigens. Antigens on the cell surface are important in cell recognition. Under normal circumstances the immune system of an individual will recognise all of his/her own cells as being 'self' owing to the familiar types of antigens present. Any cells (or other materials) with different (foreign) antigens are rapidly detected by white blood cells (leucocytes) known as **B-lymphocytes** and **T-lymphocytes** and targeted for destruction.

Types of Immune Response

An immune response, involves the recognition of foreign cells or particles and the initiation of a series of mechanisms to destroy them. There are two main types of immune response:

humoral response which is associated with B-lymphocytes and involves the production of antibodies;

cellular or cell-mediated response which is associated with the T-lymphocytes.

B-lymphocytes: The Humoral Response

On the cell surface membrane of B-lymphocytes are a number of specific antigen receptors or membrane bound antibodies. These are sites to which antigens on the surface of pathogens or other foreign material may become attached, leading to a sequence of events resulting in free antibodies being produced to destroy the foreign material.

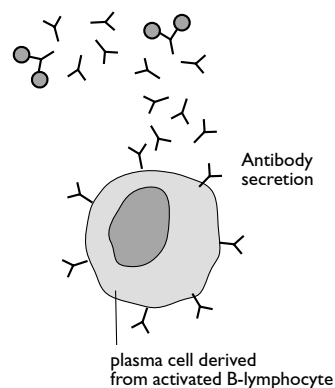
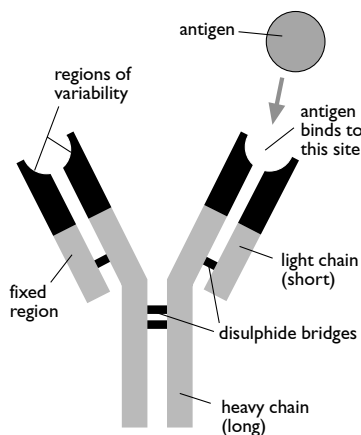
There are many thousands of specific types of B-lymphocyte and each is capable of recognising only one specific antigen. This allows highly specific antibodies to be produced in response to a vast range of antigens. For example, there is one type of B-lymphocyte which will attach to the bacterium causing TB and release antibodies against TB, and another type which attach to *Vibrio cholerae*, the bacterium responsible for cholera, and produce antibodies against it.

Antibodies are protein molecules known as immunoglobulins which are secreted by activated B-lymphocytes when a specific antigen is encountered. They are shaped like a 'Y' with the straight tail of the Y being known as the constant region since it is the same in every type of antibody. The forked part of the Y is known as the variable region and contains antigen binding sites which vary slightly in shape between each type of antibody. Antibodies do not directly destroy foreign material but target it for destruction by other branches of the immune system, including phagocytes.

Sequence of events in the humoral response against pathogens

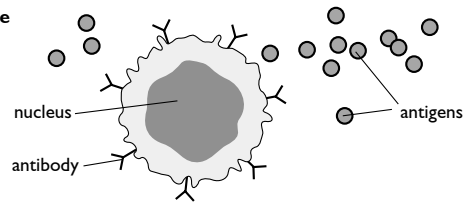
- ▼ Pathogen enters body.
- ▼ Antigens on surface of pathogen come into contact with their specific antigen receptor on a B-lymphocyte.
- ▼ Binding of antigen to antigen receptor activates the B-lymphocyte and causes it to divide by mitosis producing a clone of identical B-lymphocytes.
- ▼ Most of the B-lymphocytes turn into **plasma cells** and the rest turn into **memory cells**.
- ▼ The plasma cells begin to secrete specific antibodies against the pathogen concerned. Antibody molecules are secreted at a very high rate: up to 30 000 molecules per second.
- ▼ The antibodies attach to the antigens on the foreign material and lead to the destruction of it in one of three ways.
- ▼ By coating pathogens and causing them to stick together in such a way that they can then be engulfed by phagocytes (another type of white cell).
- ▼ By binding to the pathogens at sites which interfere with their activities. For example, by covering the protein coat of a virus and preventing it from attaching to host cells.
- ▼ By stimulating the release of other blood components called '**complement**' to destroy the pathogen.
- ▼ Once the foreign material has been destroyed, the plasma cells eventually die and antibodies stop being secreted. But the memory cells remain in the lymph nodes and the circulation. If a subsequent infection with the same pathogen occurs then some of these will turn into plasma cells and start producing antibodies. This is the basis of **natural active immunity** to diseases and underlies the practice of **immunisation** or **vaccination** which are methods of **artificial active immunity**.

Structure of an antibody

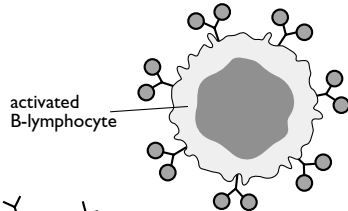


Humoral response to infection

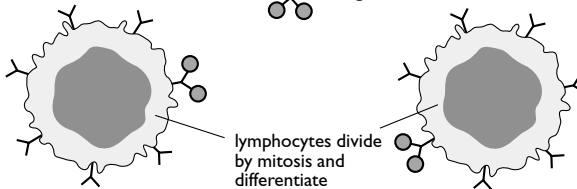
Immature B-lymphocyte



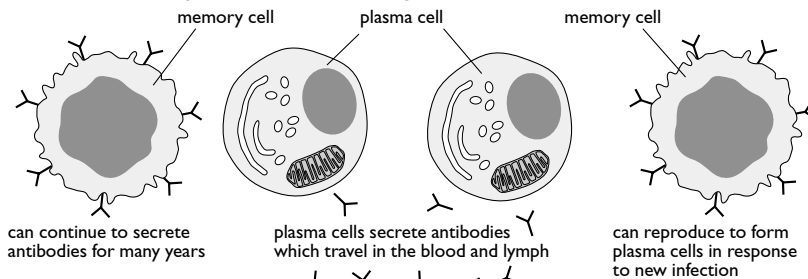
Activation



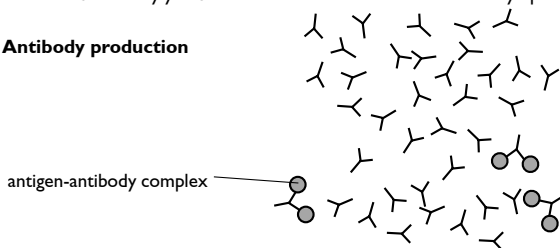
Division



Differentiation into plasma cells and memory cells



Antibody production



The cellular response of T- lymphocytes

One of the main ways in which the response of T-lymphocytes differs from that of B-lymphocytes is that T-lymphocytes do not produce antibodies but work directly on infected cells. As with B-lymphocytes, there are thousands of different types of T-lymphocyte which will each only recognise one specific antigen.

T-lymphocytes, like B-lymphocytes, have antigen receptors on their surface. However, unlike B-lymphocytes they cannot recognise antigens from pathogens on their own. They can only recognise them when they have been processed by human cells and presented on the surface of these cells. This rather complicated procedure relies on a group of special proteins called the **Major Histocompatibility Complex (MHC)** which are present on the surface of all human cells.

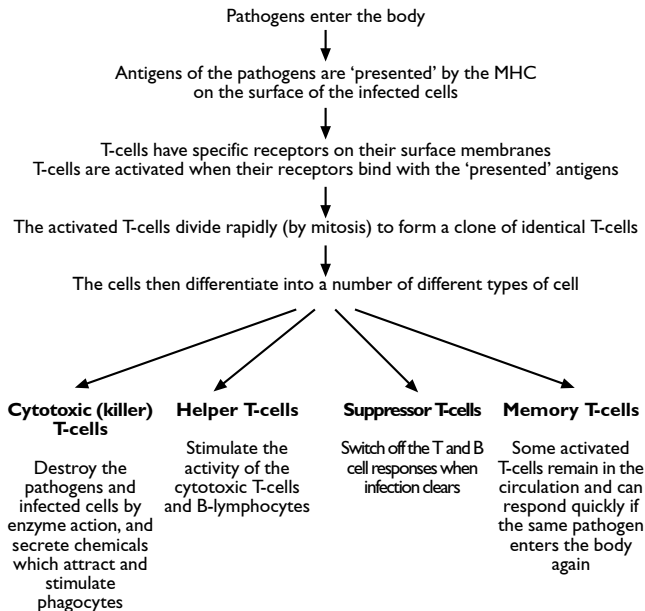
Normally the MHC allows the body's immune system to recognise its own cells and prevent their destruction. However, if a human cell becomes infected with a pathogen, then antigens from this pathogen interact with the MHC molecules and attach to them. They are now 'presented' on the surface of the cell. This alerts the body's immune system to the presence of the pathogen and allows T-lymphocytes to destroy the cell. The cell is known as an antigen presenting cell.

Antigens may also be presented by MHC molecules on the surface of B-lymphocytes and phagocytes. This is important as it allows the different parts of the immune system to cooperate with each other. Thus at the same time as producing antibodies, a B-lymphocyte can be presenting an antigen on its MHC and activating T-lymphocytes.

Sequence of events in cellular response

- ▼ Pathogen enters body.
- ▼ Antigens of pathogen which are presented by the MHC on a body cell come into contact with specific T-lymphocyte receptors which recognise them.
- ▼ T-lymphocyte binds to presented antigen on infected cell via antigen receptor.
- ▼ T-lymphocyte is activated and divides to form a clone of identical T-lymphocytes which enter the circulation and can attach to other infected cells.
- ▼ T-lymphocytes destroy the the pathogens and the cells to which they are bound. Some do this by releasing lytic enzymes into the infected human cells. Others release chemical messengers called cytokines which attract phagocytes to engulf the cells.
- ▼ Some T-lymphocytes then turn into memory cells which remain in circulation and in the lymph nodes in case of further infection.

Cellular Response to infection T-Lymphocytes (T-cells)



Helper and suppressor T-lymphocytes

The T-lymphocytes described above are called **cytotoxic T-lymphocytes**. There are also two other types of T-lymphocyte which work in different ways. **Helper T-lymphocytes** 'help' B lymphocytes and cytotoxic T-lymphocytes to function by releasing chemicals called cytokines and interleukins which stimulate the activity of the lymphocytes. They are only able to do this once they have been activated by binding to an antigen presented on an antigen presenting cell. It is these cells which are destroyed by the HIV virus (section 12.1). **Suppressor T-lymphocytes** are important in switching off the B and T-lymphocyte responses to a particular pathogen once the infection has been cleared.

◆ CHECKPOINT SUMMARY

- ◆ Antigens are defined as molecules, which, when foreign to an individual, stimulate the production of antibodies by B-lymphocytes.
- ◆ Antibodies are protein molecules known as immunoglobulins which are secreted by activated B-lymphocytes when a specific antigen is encountered.
- ◆ There are two main types of immune response: the Humoral Response which is associated with B-lymphocytes and involves the production of antibodies, and the Cellular or cell-mediated response which is associated with the T-lymphocytes.
- ◆ Binding of antigen to antigen receptor activates the B-lymphocyte and causes it to divide by mitosis producing a clone of identical B-lymphocytes.
- ◆ Most of the B-lymphocytes turn into plasma cells and the rest turn into memory cells.
- ◆ The plasma cells begin to secrete specific antibodies against the pathogen concerned.
- ◆ Once the foreign material has been destroyed, the plasma cells eventually die and antibodies stop being secreted. But the memory cells remain in the lymph nodes and the circulation.
- ◆ If a subsequent infection with the same pathogen occurs then some of these will turn into plasma cells and start producing antibodies. This is the basis of natural active immunity to diseases and underlies the practice of immunisation or vaccination which are methods of artificial active immunity.
- ◆ T-lymphocytes do not produce antibodies but work directly on infected cells.
- ◆ Antigens of pathogen which are presented on a body cell come into contact with specific T-lymphocyte receptors which recognise them.
- ◆ T-lymphocyte binds to presented antigen on infected cell via antigen receptor.
- ◆ T-lymphocyte is activated and divides to form a clone of identical T-lymphocytes which enter the circulation and can attach to other infected cells.
- ◆ T-lymphocytes destroy the pathogens inside the cells to which they are bound. Some do this by releasing lytic enzymes into the infected human cells. Others release chemical messengers called cytokines which attract phagocytes to engulf the cells.
- ◆ Some T-lymphocytes then turn into memory cells which remain in circulation and in the lymph nodes in case of further infection.

Primary and secondary immune responses

A primary immune response is the type of response which occurs when a pathogen is encountered by the body for the first time. A secondary response is that which occurs on a second or subsequent encounter with the same pathogen.

Both B-lymphocytes and T-lymphocytes can form memory cells after encountering a pathogen in the body on one occasion. The function of these is to allow the body to 'remember' pathogens it has encountered before and produce a much faster immune response to them the second time around.

T-lymphocyte memory

When a familiar antigen is encountered, T-lymphocyte memory cells will divide to form functional T-lymphocytes, enter the circulation and move to the area of infection. They are known to move to the area of the body where the specific infection occurred before. Some memory cells always remain in case of future infections.

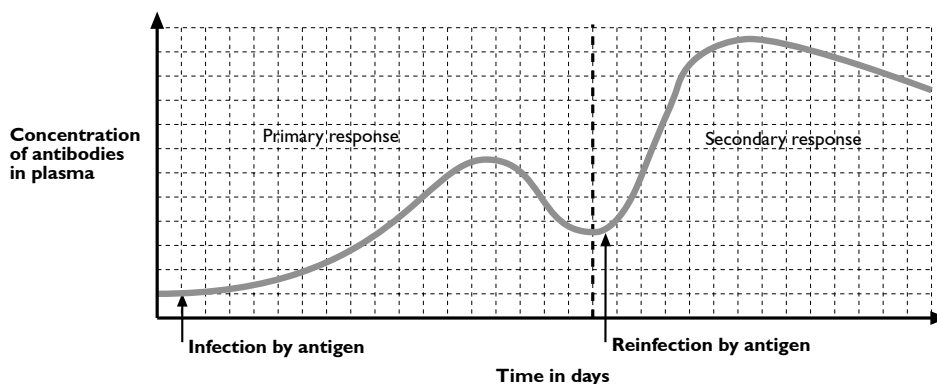
B-lymphocyte memory

B-lymphocyte memory cells produced during a primary encounter with a pathogen will divide and form new antibody producing plasma cells when they encounter their familiar pathogen again. Some memory cells remain in case of future infections.

Two main differences exist between the primary and secondary responses to a pathogen by B-lymphocytes.

- ▼ Antibodies are produced more rapidly in the secondary response.
- ▼ Larger amounts of antibodies are produced in the secondary response.

Primary and secondary immune response



The significance of memory cells

The presence of memory cells means that with some diseases such as mumps or chicken pox, one infection can make a person immune to that disease for the rest of their life. The pathogens causing these diseases will almost certainly get into their body again during that time, but the secondary immune response is so rapid that they will not become ill. With other infections, such as malaria, and influenza, the immunological memory is not so good and people can suffer many bouts of illness from the same type of pathogen. This is because some pathogens have a very high mutation rate in the genes coding for antigen proteins. The structure of the antigens changes slightly and the memory cells fail to recognise it.

Types of immunity: Active and Passive

The immunity to a disease which follows a natural infection with a pathogen (as described above) is known as **natural active immunity**. It involves a pathogen entering the body and allowing memory cells to be formed for future protection against the same disease. The person does not necessarily have to get ill from the infection for the immune system to work in this way.

In addition to this natural immunity there are two main ways in which immunity can be provided artificially. This is known as **immunisation** and includes vaccination (which produces **artificial active immunity**, often for life) and the provision of antibodies (**artificial passive immunity**) which provides immunity for a short period of time only.

Vaccination: artificial active immunity

Vaccination is a process by which immunity to a disease is achieved without experiencing a natural infection with that disease. It works on the same principle as natural active immunity, but instead of a person becoming infected with a pathogen via the normal routes, they are given a very small dose of antigens of the pathogen to which immunity is required via an injection or an oral vaccine. The different types of vaccine available are discussed below.

Following immunisation, B-lymphocytes are stimulated by antigens on the pathogen, producing plasma cells and hence antibodies, and memory cells. T-lymphocytes may also be stimulated and produce memory cells. These memory cells remain in the body and allow the person to respond quickly if they encounter the same pathogen again (during a natural infection), preventing them from becoming ill. This type of immunity is known as **artificial active immunity**, since the pathogen is introduced to the body in an artificial manner and the person does not usually get ill from the disease as they often do in natural active immunity. Some vaccines provide complete immunity after a single dose. Others require a second dose known as a 'booster' to allow a greater number of memory cells to form.

Vaccination is a safe and effective way of gaining immunity to diseases which might otherwise cause serious illness or death if caught naturally.

Types of vaccine

There are many different types of vaccine which have been developed to ensure that vaccinations are both effective and safe. It is usually unsafe to use a live natural pathogen, as even if given in small quantities, these may multiply rapidly and actually cause disease. Other options include:

- ▼ Vaccines containing a 'toxin' produced by the pathogen but not the whole pathogen. e.g. diphtheria.
- ▼ Vaccines containing dead (heat killed) pathogens, e.g. influenza. Even though the organism is dead its antigens can still be recognised and an immune response made against them.
- ▼ Vaccines containing a modified or weakened (attenuated) form of the pathogen. e.g. polio, *Rubella*, TB. In this case the microorganism is alive but has been modified in a way which prevents it from being harmful. It may have been treated with heat or chemicals to slow its rate of growth. Alternatively, naturally occurring mutant forms of the organism which have the same antigens, but do not cause disease may be used.
- ▼ Genetically engineered vaccines. Genetic engineering technology is allowing the development of new, safer and more effective vaccines. This is focussed on the identification of the genes of pathogens, and their removal, alteration or replacement.
- ▼ Genetically engineered vaccines may thus contain live pathogens which have been genetically modified by removing or 'switching off' potentially harmful genes. An example is the new cholera vaccine which is being developed from a cholera bacterium in which the two toxin genes have been removed. This should be much more effective than the current vaccine which uses dead cholera bacteria.
- ▼ In other instances the gene or genes coding for the antigenic proteins on the surface of a pathogen may be isolated, removed from the pathogen, and inserted into another form of bacterium or yeast. This can allow for the microbial production of vast amounts of antigen for use in vaccines. For example, the hepatitis B vaccine is a recombinant yeast vaccine. Hepatitis antigens are produced by yeast cells which contain a plasmid into which one of the viral genes responsible for antigen production has been inserted.

PASSIVE IMMUNITY

This is the process whereby ready made antibodies are given to a person to offer them rapid but short-term immunity to a disease. It is called passive because the person plays no role in actually producing the antibodies against that disease. Since memory cells are not given, such passive immunity will only last a few weeks or months, after which time the antibodies will be broken down by the body and stop working. There are two types of passive immunity.

Natural passive immunity occurs when antibodies are passed from mother to fetus via the placenta or from mother to baby via the colostrum (first milk). These antibodies protect the baby from diseases which it may encounter in the first few months of life before its own immune system starts functioning properly.

Artificial passive immunity occurs when antibodies produced in another organism are injected into a person to give them short term

protection against a specific disease. Antibodies may be given to a person who has already got the disease (such as someone who has got rabies from an infected dog bite) or to a person who is at risk of catching a disease such as tetanus. In the past, such antibodies could only be obtained from the blood of other animals who had been deliberately infected with the pathogen.

Now some of the antibodies required for passive immunisation are produced as **monoclonal antibodies** in the laboratory. These are antibodies produced by a single clone of identical B-lymphocytes (plasma cells). They are produced by cells known as **hybridomas** which are created in the laboratory by fusing a plasma cell of the desired type (one that produces antibodies against a specific pathogen) with a cancer cell (myeloma). The hybridomas have the ability to constantly divide, like a cancer cell, and to produce antibodies like a plasma cell. The monoclonal antibodies produced have a variety of uses in medicine, research and diagnostic tests. (section 12.9).

◆ CHECKPOINT SUMMARY

- ◆ Passive immunity occurs when the individual plays no role in actually producing the antibodies against that disease. Since no memory cells are formed, such passive immunity will only last a few weeks or months, after which time the antibodies will be broken down by the body and stop working.
- ◆ Natural passive immunity occurs when antibodies are passed from mother to fetus via the placenta or from mother to baby via the colostrum (first milk). These antibodies protect the baby from diseases which it may encounter in the first few months of life before its own immune system starts functioning properly.
- ◆ Artificial passive immunity occurs when antibodies produced in another organism are injected into a person to give them short term protection against a specific disease.
- ◆ Antibodies required for passive immunisation can be produced as monoclonal antibodies in the laboratory.
- ◆ The immunity to a disease which follows a natural infection with a pathogen is known as natural active immunity.
- ◆ Immunisation includes vaccination (which produces artificial active immunity, often for life) and the provision of antibodies (artificial passive immunity) which provides immunity for a short period of time only.
- ◆ Vaccination is a process by which immunity to a disease is achieved without experiencing a natural infection with that disease. A very small dose of antigens of the pathogen to which immunity is required is given via an injection or an oral vaccine.
- ◆ Vaccines containing a 'toxin' produced by the pathogen but not the whole pathogen. e.g. diphtheria.
- ◆ Vaccines containing dead (heat killed) pathogens. e.g. influenza. Even though the organism is dead its antigens can still be recognised and an immune response made against them.
- ◆ Vaccines containing a modified or weakened (attenuated) form of the pathogen. e.g. polio, Rubella, TB.
- ◆ Genetically engineered vaccines may contain live pathogens which have been genetically modified or attenuated by removing or 'switching off' potentially harmful genes. Or the gene or genes coding for the antigenic proteins on the surface of a pathogen may be removed from the pathogen, and inserted into another form of bacterium or yeast. This can allow for the microbial production of vast amounts of antigen for use in vaccines.

4

Cell division

Content

Genetic information is passed from cell to cell during division

Mitosis

- ▼ The process of mitosis emphasising the behaviour of chromosomes, the role of the spindle and the genetic identity of the products.
- ▼ The use of appropriate staining techniques in the study of mitosis in suitable plant material (see practical work).

The cell cycle

- ▼ Mitosis and the cell cycle.
- ▼ The relationship between DNA replication and the events of the cell cycle.

Meiosis

- ▼ The importance of meiosis in halving the chromosome number in gametes so that, after fertilisation, the diploid chromosome number is restored in the resulting zygote.

MITOSIS

New cells (daughter cells) are formed by cell division. Cell division is initiated by the nucleus, and nuclear division is the first visible sign of the process of cell division. Mitosis is the commonest form of nuclear division, and results in two genetically identical daughter nuclei being formed. Mitosis occurs in the cell division seen in growth, repair, the replacement of cells which have a limited life span like our red blood cells and skin cells, and in asexual reproduction in many plants and lower animals. In the case of single celled organisms this type of asexual reproduction is called binary fission. The development of a multicellular organism from a fertilised egg by mitosis, means that all the cells of the body are genetically identical to the fertilised egg. The development of an organism from the fertilised egg is the story of the controlled expression of this genetic information, with only a small fraction of the genes being expressed in cells as they differentiate to form tissues specialised for specific (and limited) functions. In mature cells most of the DNA is inactive all of the time.

Asexual reproduction does not involve the fertilisation of gametes, and as a result of mitosis produces offspring which are genetically identical to each other (clones). Many multicellular organisms, especially plants can reproduce their entire bodies in this way, either from specialised structures, or single celled spores.

The production of genetically identical cells in the growth and development of multicellular organisms allows certain cells to retain the ability to develop into any other type if needed as a result of some damage. For example, stem cells in mammals can divide to form any of the different types of blood cell. In the asexual reproduction of organisms mitosis allows for the rapid exploitation of optimal conditions, where any variation would be disadvantageous.

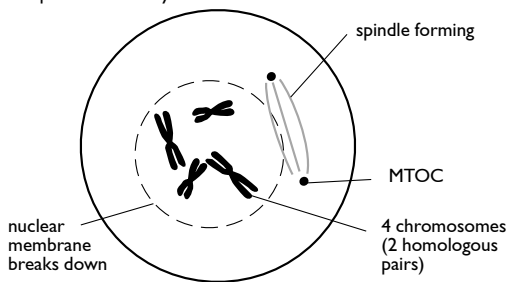
The main stages of mitosis

Chromosomes only form and become visible (when stained and viewed under the light microscope) when the nucleus of a cell is just about to divide. This is because only at this time is the DNA/histone mixture wound up (condensed) as tightly as it can be. By this stage the DNA has also copied itself (replicated) so each chromosome appears as a double structure, joined at one point (the centromere). Whilst they remain joined in this way the two daughter chromosomes are referred to as chromatids.

Nuclear division (mitosis) occurs in four main stages, namely prophase, metaphase, anaphase and telophase.

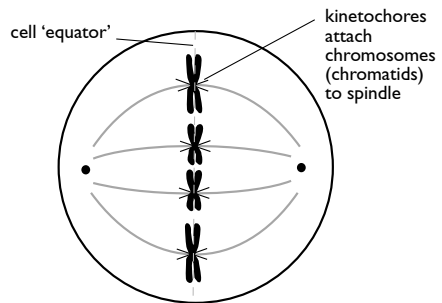
1 Prophase

Cell is rounded
MTOC's migrate to opposite poles
nuclear membrane disintegrates
spindle formed by MTOCs



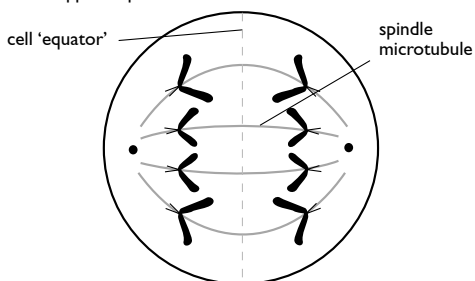
2 Metaphase

Chromosomes held on 'equator' of cell under tension. MTOCs at opposite poles



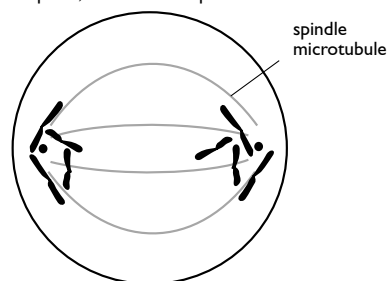
3 Anaphase

Chromatids separate and move towards opposite poles



4 Telophase

Chromosomes form beginnings of new nuclei at opposite poles, nuclear envelope reforms



Prophase is the first phase of mitosis, and as it begins, the DNA has already replicated, all protein synthesis has stopped, and each chromosome is condensed into the characteristic double chromatid form. At the same time the cell rounds up, the nuclear membrane breaks down into small fragments, and a spindle of microtubules forms between two microtubule organising centres (MTOCs) at opposite poles of the cell.

Metaphase is defined as when the chromosomes become attached to the spindle by structures called centromeres (kinetochores). Kinetochore consist of microtubules and 'motor' proteins which utilise ATP energy to pull on the spindle. The effect of both sets of kinetochores pulling in opposite directions is that the chromosomes line up along the middle (equator) of the spindle.

Anaphase occurs when quite suddenly, and all at once, the kinetochores and chromatids of each double chromosome separate, with each chromatid (now daughter chromosome) being pulled under tension in opposite directions towards the poles of the cell by the kinetochores, with their arms trailing in the characteristic 'V' shape.

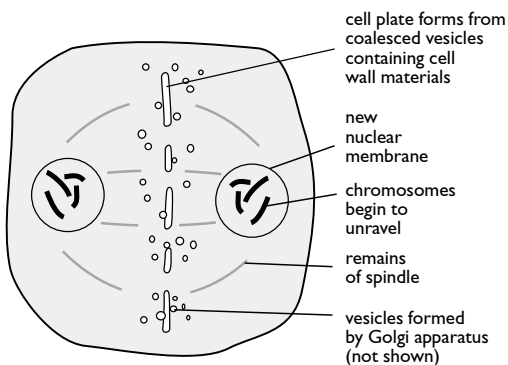
Telophase is the stage when the two new, identical sets of chromosomes reach the opposite poles and a new nuclear envelope forms to surround each set.

Nuclear division by mitosis is followed by division of the cytoplasm into two equal parts. This process is called **cytokinesis** and it occurs differently in animals and plants.

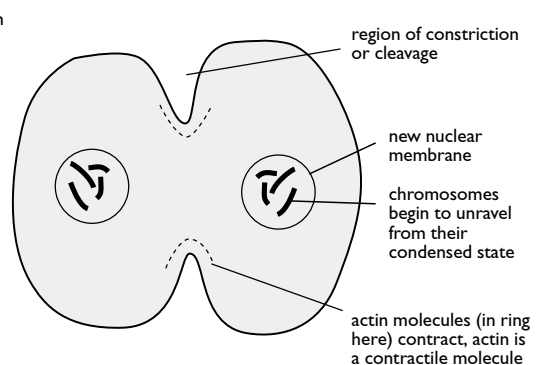
In animal cells the spindle disintegrates during telophase and the microtubule components reassemble into a new cytoskeleton. The equator of the cell is constricted by a ring of contractile proteins (actin) in the process of cleavage, to create two roughly equal cells.

In plant cells the spindle lingers a little longer and seems to act as a guide to bring Golgi apparatus and associated secretory vesicles to the equator. The vesicles deposit their contents, which are the building constituents of a new cell wall, on the equator to form a plate of deposited material (the cell plate). Some of the vesicles remain intact and make connecting channels through the new cell wall (plasmodesmata).

Cytokinesis in plant cells



Cytokinesis in animal cells



THE CELL CYCLE

Interphase refers to the greater part of the life of a cell when there is no apparent activity. The period of mitosis and cytokinesis occupy only a fraction of the entire life of a cell. Together, interphase, mitosis and cytokinesis make up the **cell cycle**. The term interphase should not be interpreted to mean that the cell is resting. Interphase can itself be divided into three distinct periods called G1, S and G2. ('G' refers to the 'gaps' between DNA replication and mitosis.)

G1 is by far the longest period of the cell cycle. During G1, the cell machinery carries out protein synthesis, and growth occurs. All the cells of the body have identical sets of genes in their nuclei but the cells develop in different ways to perform different functions. This development process is called cell differentiation in which different genes are switched on and off, so that only one small part of the genetic code is active in each cell.

After a period of growth and differentiation, the length of which depends upon a variety of internal and external factors, protein synthesis is suspended, and the DNA set replicates itself. This is the **S** phase. DNA replication is followed by another 'gap' period, **G2**, before mitosis starts, in which the cytoskeleton of the cell breaks down and the microtubule components begin to reassemble into spindle parts. Without a cytoskeleton, the cell assumes a more spherical shape (see prophase).

The timing of events within the cell cycle is extremely variable. In ideal conditions, where food and oxygen are in plentiful supply and temperature and pH values are optimal, bacterial cells can complete the cycle to produce a new generation every 20 minutes. Other cells, for example muscle cells, never complete the cycle, a condition called terminal differentiation.

It has become clear that the onset of the S (DNA replication) phase and the M (mitotic) phase are determined by genes which are very similar in all organisms. These genes direct the synthesis of enzymes which in turn activate the enzymes initiating S and M phases.

The rate of cell division can be controlled by naturally occurring plant substances which affect the process of nuclear spindle formation. These include vincristine and vinblastine (from the periwinkle plant *Catharanthus rosea*). Both of these substances are used to control cancers such as leukemia and lymphoma. They work, at a molecular level, by binding on to the spindle microtubules, preventing spindle assembly.

The plant substance, taxol, from the Pacific Yew (*Taxus brevifolia*) has almost the reverse effect on spindle formation, causing an over production of microtubules which effectively stops cell division. Taxol has been used to treat ovarian cancers. As more is known about the factors controlling the cell cycle, new possibilities will open up for drug therapy which can be more specifically targeted. Work in this field will be aided by the research which has discovered the sequence of bases in the DNA of the human chromosomes (the Human Genome Project) as it reveals more and more about the predisposition of individuals with particular gene combinations to cancer.

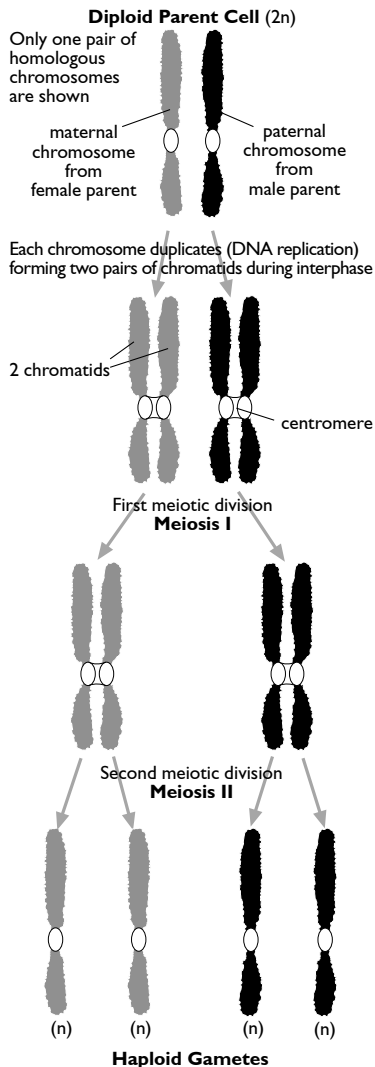
◆ CHECKPOINT SUMMARY

- ◆ Mitosis is nuclear division in which the daughter nuclei have the same chromosome number as the parent nucleus, typically diploid nuclei producing diploid daughter nuclei.
- ◆ The daughter nuclei are therefore genetically identical to the nucleus of the cell that produced them (except for any mutations that may occur).
- ◆ Mitotic cell division is seen in growth, repair and asexual reproduction.
- ◆ As a result of mitosis in the growth of an organism from the fertilised egg all cells therefore are genetically identical (totipotent).
- ◆ Similarly, offspring of asexual reproduction lack genetic variation.
- ◆ The production of genetically identical cells retains the full set of genetic information needed for the replacement of any cells in repair processes, and in the asexual reproduction of organisms it allows for the rapid exploitation of optimum conditions where any variation would be disadvantageous.
- ◆ The DNA replicates in interphase so that in the first stage of mitosis (prophase) the chromosomes appear as double structures of two chromatids (daughter chromosomes) joined at the centromere.
- ◆ The nuclear spindle apparatus (NSA) forms from the dissolution of the nuclear membrane and the centrioles replicate and migrate to either pole of the NSA.
- ◆ The centromeres align on the equator of the NSA (metaphase).
- ◆ The centromeres divide and the chromatids are moved to the opposite poles (anaphase).
- ◆ Two new daughter nuclei are formed (telophase).
- ◆ The cytoplasm is divided between two daughter cells by cleavage in animal cells and the laying down of new cell walls in bacteria and plants
- ◆ The formation of two new cells completes the cell cycle.

MEIOSIS

Meiosis is a special type of cell division called a **reduction division**. Meiosis occurs during the production of sex cells (gametes) in animals, and in spore formation which precedes gamete production in plants. Meiosis results in the number of chromosomes being halved from the normal **diploid** to the **haploid** number. The mechanisms of cell division are very similar to that already described for mitosis, but meiosis involves two divisions, referred to as the first and second divisions (meiosis I and meiosis II), and the result of meiosis is four cells (gametes or spores) each containing half the original number of chromosomes.

Meiosis involves certain genetic 'mixing' events, and these, along with the random fusion of the gametes in fertilisation to form a diploid zygote, result in genetic variation in the offspring which may be of adaptive advantage.



◆ CHECKPOINT SUMMARY

- ◆ Each gamete of a sexually reproducing organism contains one set of chromosomes (haploid).
- ◆ When two such gametes fuse in fertilisation they produce a zygote with two sets of chromosomes (diploid).
- ◆ Chromosomes in a diploid nucleus thus occur in pairs, each member of a pair carrying the same genes in the same relative positions (homologous chromosomes).
- ◆ Slight variations (alleles) of a gene at a particular locus can arise by mutation, so that an organism can either have an identical pair of genes at a locus (homozygous) or a pair that differs slightly (heterozygous).
- ◆ Pairs of sex determining chromosomes are only partially homologous e.g. the human Y chromosome has a short non-homologous arm which carries the male determining genes.
- ◆ The production of haploid gametes from a diploid cell requires a reduction division (meiosis) in which the chromosome number is halved from diploid to haploid.
- ◆ The production of haploid gametes ensures the maintenance of the diploid chromosome number in subsequent generations.

5

DNA and protein synthesis

Contents

DNA as genetic material

- ▼ The structure of DNA, mRNA and tRNA in terms of nucleotides, base pairing and hydrogen bonding.
- ▼ Evidence that DNA is the genetic material

Nucleic acids and DNA replication

- ▼ The roles of nucleic acids in coding information, protein synthesis and the replication of DNA
- ▼ The semi-conservative replication of DNA

Protein synthesis

- ▼ The genetic code as a non-overlapping, degenerate code. Introns as non-coding DNA.
- ▼ The mechanism of protein synthesis involving the roles of mRNA, tRNA and the ribosomes.
- ▼ DNA determines the structure of enzymes which determine the phenotype of an organism, illustrated by reference to cystic fibrosis and phenylketonuria

DNA AS GENETIC MATERIAL

The structure of DNA and RNA

DNA (deoxyribo(se)nucleic acid), is so named because it is an acid and it is found in the nucleus. It is a polymer made up of repeating sub-units, in this case, nucleotides linking up to form a polynucleotide. What makes DNA so important and so unique is that it contains within its structure the genetic code, and it can produce copies of itself (replicate).

Evidence that DNA is the genetic material

The nucleus of cells contains mostly DNA and protein. For many years it was thought that protein was the hereditary material, but when it became possible to extract and measure the quantity of DNA, it was found that the DNA content doubled in cells just before dividing. It was also found that, in the formation of sex cells, the amount of DNA halved. Both pieces of evidence underline its central role in inheritance. Now it has become possible to analyse the molecular structure of DNA, to unravel the chemical code it contains, and to transfer pieces of this code between one organism and another in the process known as genetic engineering.

DNA is a polynucleotide made up of repeating nucleotide units. A **nucleotide** consists of three main parts: a pentose (5 carbon) sugar,

joined to a phosphate group, and a nitrogen-containing organic base. There are four different types of nucleotide in DNA each with a different organic base. The bases are **adenine** (A) and **guanine** (G) which belong to a group of chemicals called **purines**, and **cytosine** (C) and **thymine** (T) which belong to group called **pyrimidines**. Chains of nucleotides link up by condensation reactions, with the sugar and phosphate molecules forming the 'spine' of each strand of the molecule, with the bases to one side.

DNA consists of two such chains or strands linked by **hydrogen bonds** which form between pairs of bases. As the hydrogen bonds form, so the two strands of the DNA molecule twist into the spiral structure known as the double helix. Hydrogen bonds form only between specific pairs, namely A with T and C with G. The strands are therefore **complementary** to each other. The sequence along one strand determines the sequence of the other (complementary) strand. The understanding of the structure of the DNA double helix was one of the most significant breakthroughs in Biology.

RNA (Ribo(se)nucleic acid), like DNA, is a polymer made up of repeating nucleotides. It differs from DNA in the following three ways.

RNA is single stranded, not double stranded.

The pentose sugar in RNA is ribose, not deoxyribose (molecules of ribose contain an additional oxygen atom).

The pyrimidine base Uracil (U) is found instead of Thymine (T).

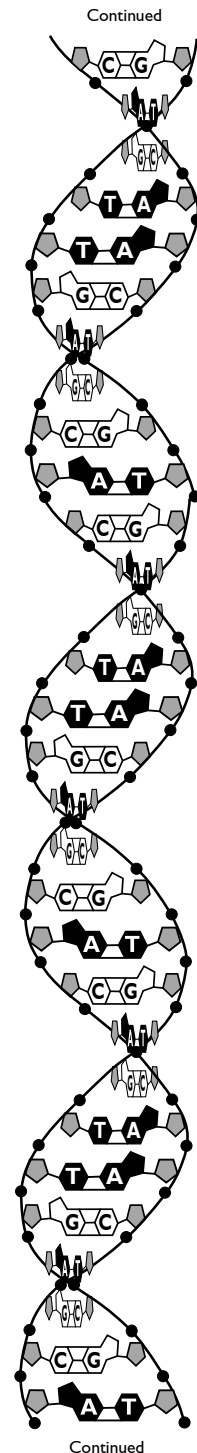
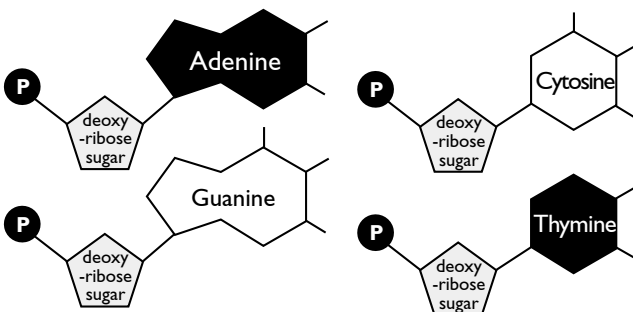
Living cells produce three different types of RNA in their nuclei, each designed to carry out a specific function in protein synthesis.

Messenger RNA (mRNA) consists of a single strand, between 75 and 3000 nucleotides long, produced in the nucleus as an exact copy of a selected section of the DNA code. The mRNA molecules travel out of the nucleus to the ribosomes where proteins are synthesised.

Transfer RNA (tRNA) is a shorter length of RNA, between 75 and 90 nucleotides long, twisted into a characteristic shape which allows one end to attach to a specific amino acid, and the other to expose a triplet of bases called the anti-codon which attaches to mRNA at the site of protein synthesis. tRNA molecules bring amino acids to their correct position as the proteins are being assembled. Each type of amino acid is carried by a different type of tRNA molecule with a specific anti-codon.

Ribosomal RNA (rRNA) consists of more than 1000 nucleotides and is found in ribosomes which are composed of an approximately equal mixture of proteins and rRNA. Ribosomal RNA is manufactured within the nucleolus.

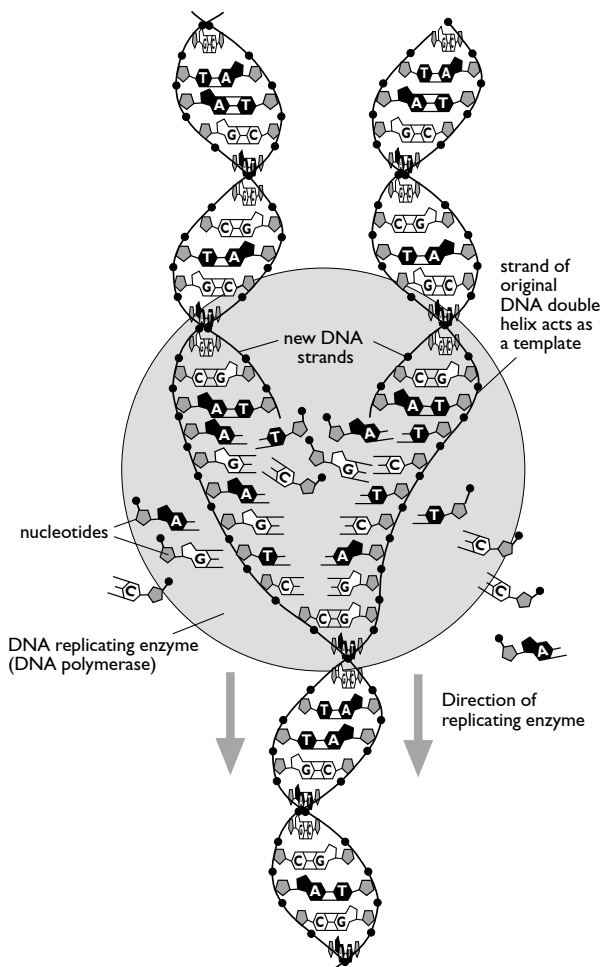
The Nucleotides of DNA



Replication of DNA

In the process of DNA replication the original strands separate, and each acts as a pattern (template) for the formation of another new strand of DNA. Each of the daughter DNA molecules retains (conserves) one of the original strands and has one new one. For this reason, the process of replication is said to be **semi-conservative**. The process requires a supply of the four different nucleotides, energy in the form of ATP, and an enzyme called DNA polymerase, which is required to catalyse the condensation reactions by which free nucleotides form a copy of the template strand.

Replicating DNA enzyme



◆ CHECKPOINT SUMMARY

- ◆ The nucleus of cells contains mostly DNA and protein.
- ◆ The DNA content doubles in cells just prior to dividing.
- ◆ It was also found that, in the formation of gametes (sperms and eggs), the amount of DNA halved. This evidence underline its central role in inheritance.
- ◆ Now it has become possible to analyse the molecular structure of DNA, to unravel the chemical code it contains, and to transfer pieces of this code between one organism and another in the process known as genetic engineering.
- ◆ DNA and RNA are long chain polynucleotide molecules with repeating sub-units of nucleotides.
- ◆ Nucleotides consist of a 5 carbon sugar; a phosphate and an organic base.
- ◆ In DNA the sugar is deoxyribose and the organic base can be one of adenine, thymine, cytosine and guanine.
- ◆ In RNA the sugar is ribose and the organic base can be one of adenine, uracil, cytosine and guanine.
- ◆ The DNA molecule is a double stranded helix with the two strands held together by hydrogen bonds between complementary base pairs adenine-thymine and cytosine-guanine.
- ◆ RNA is a single stranded helix with base pairs of adenine-uracil and cytosine-guanine.
- ◆ There are three types of RNA - messenger; transfer and ribosomal.
- ◆ DNA carries out semi-conservative replication in which each strand of the original molecule acts as a template for the construction of a new one, so that each new double stranded molecule of DNA consists of one original strand and a new complementary strand.

Experimental evidence for the semi-conservative mechanism of DNA replication includes experiments in which the mass of the DNA content of *E. coli*, grown on different nutrient media was determined and compared. One batch of microorganisms was cultured on a medium containing a 'heavy' isotope of nitrogen (^{15}N), the DNA was extracted and compared with that of organisms cultured on a medium containing normal 'light' nitrogen (^{14}N). The *E. coli* cells grown on 'heavy' nitrogen were then transferred to a new medium containing only light nitrogen and allowed to divide once to produce a new generation. The DNA of these daughter cells was found to be halfway between the 'heavy' and 'light' values, indicating that half the DNA was old and half was new (semi-conservative replication).

The genetic code

The DNA base sequence forms a four letter language which, as you have seen, can be copied every time a cell divides. This DNA base sequence determines the sequence of amino acids in a polypeptide chain (the primary structure of proteins). A sequence of bases which codes for one polypeptide is known as a gene. Each gene codes for a different polypeptide chain. The primary structure of a polypeptide is the basis of the particular structural shape and characteristic of the final protein, e.g. enzymes, structural materials and membrane proteins. The base code determines the structure, functions and individuality of every organism.

Remember that up to twenty different types of amino acids may occur in a single protein. The DNA code has a four letter base language (A,G,C,T). A triplet of bases (sequence of three in a row) codes for each amino acid, for example 'CGC' codes for the amino acid alanine (Ala) whilst 'GCT' codes for arginine (Arg), so the code

Amino acid	Abbr.	mRNA codons	Essential
Alanine	Ala	GCA, GCC, GCG, GCU	
Arginine	Arg	AGA, AGG, CGA, CGC, CGG, CGU	infants only
Asparagine	Asn	AAC, AAU	
Aspartic acid	Asp	GAC, GAU	
Cysteine	Cys	UGC, UGU	
Glutamic acid	Glu	GAA, GAG	
Glutamine	Gln	CAA, CAG	
Glycine	Gly	GGA, GGC, GGG, GGU	
Histidine	His	CAC, CAU	infants only
Isoleucine	Ile	AUA, AUC, AUU	E
Leucine	Leu	CUA, CUC, CUG, CUU, UUA, UUG	E
Lysine	Lys	AAA, AAG	E
Methionine	Met	AUG (Start codon)	E
Phenylalanine	Phe	UUC, UUU	E
Proline	Pro	CCA, CCC, CCG, CCU	
Serine	Ser	AGC, AGU, UCA, UCC, UCG, UCU	
Threonine	Thr	ACA, ACC, ACG, ACU	E
Tryptophan	Trp	UGG	E
Tyrosine	Tyr	UAC, UAU	
Valine	Val	GUA, GUC, GUG, GUU	E
STOP CODONS		UAA, UAG, UGA	

'CGC-GCT-CGC' would be translated as 'Ala-Arg-Ala' in a polypeptide. Note that the code is **non-overlapping**, i.e. it must be read **CGC-GCT-CGC**, not **CGC-GCG-CGC-GCT-CTC** etc.

Sixty four different triplet codes (codons) are possible with this four letter base language, more than sufficient to code for the twenty different types of amino acids. The term **degenerate** is applied to the genetic code because there are more codons available than can be used to code for amino acids. In fact most amino acids are coded for by more than one codon each. The process by which this code is used in protein synthesis, is described below.

Protein Synthesis

Protein synthesis involves all three of the RNA types described above working in combination, and is achieved in two stages. As the DNA cannot leave the nucleus, first the code has to be read and copied (transcribed) into mRNA, in a process called **transcription**. The mRNA moves out of the nucleus and associates with the ribosomes, where the code (now embedded in the mRNA) is used in the synthesis of a polypeptide in the process of translation.

Transcription begins as an enzyme, **RNA polymerase**, attaches to the **'Start' codon** of a gene. The DNA base sequence of this 'Start' codon is always 'TAC'. As RNA polymerase attaches to the DNA, the hydrogen bonds binding the two strands of the DNA double helix break apart to expose the sequence of triplet base codons on both strands. The enzyme then travels rapidly down the length of the gene like a zip fastener unzipping the DNA. As it does so, a molecule of mRNA is formed copying one of the DNA strands. Only one of the two DNA strands (the template strand) is copied into mRNA. The mRNA is therefore an exact replica (save that 'U' replaces 'T') of the other strand, consequently referred to as the **copy strand**. When the enzyme reaches the 'Stop' codon of the gene, transcription ends, the enzyme is free to start the process again, and mRNA is released out through a pore in the nuclear membrane to the ribosomes where the next stage occurs.

A complicating feature of the process of transcription in eukaryotic cells (cells with a nucleus) is that the length of DNA to be copied into mRNA (the gene) has sections of **'junk code'** which do not code for any of the required amino acids in a polypeptide chain. The 'junk' sequences are called **introns** (because they stay within the nucleus) and the parts to be transcribed and translated are called **exons**. The whole length is transcribed into mRNA, but before it is released from the nucleus, the introns are cut out with the assistance of enzymes, leaving a single, 'unpolluted', strand.

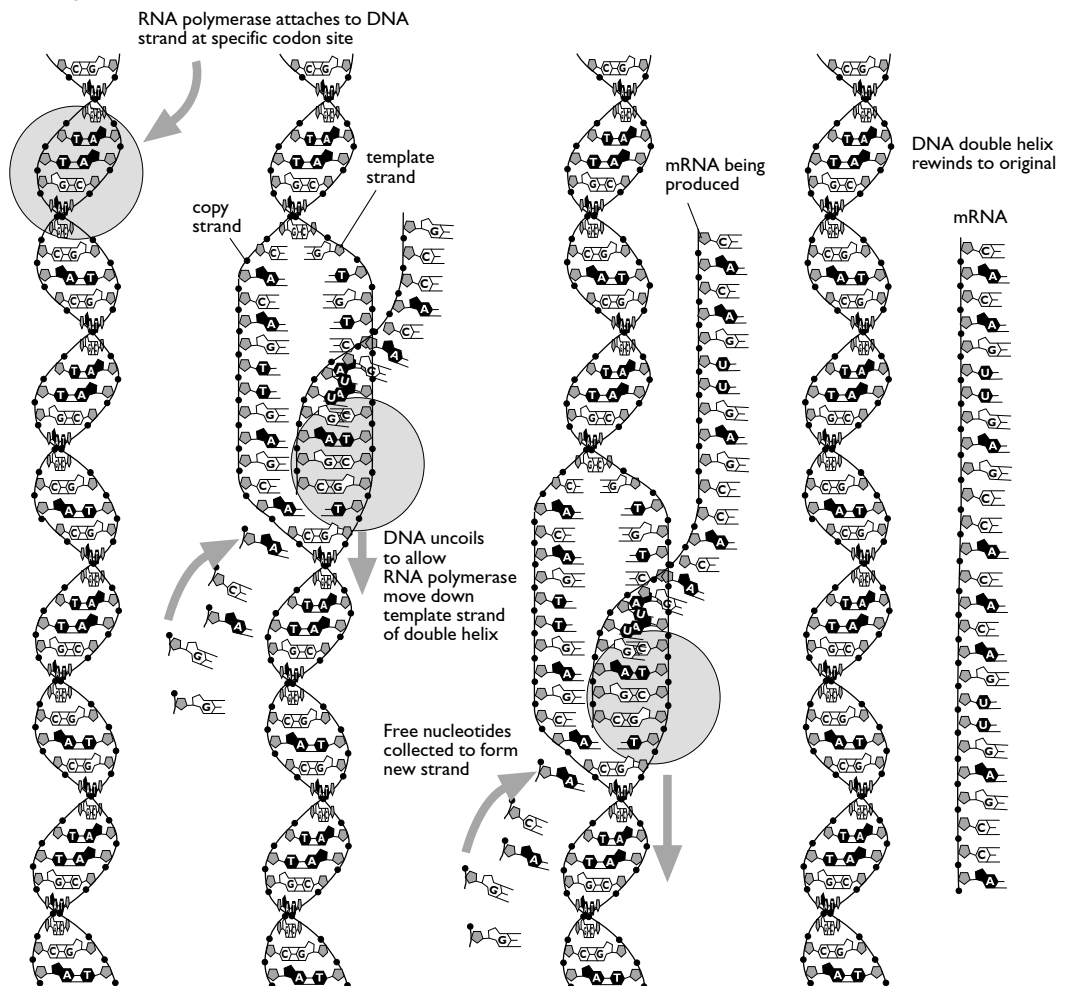
Translation occurs at ribosomes which are made up of two sub-units, one larger than the other, which come together only for the purpose of translating a length of mRNA. The mRNA binds to the small sub-unit at its 'Start' codon (AUG). It is then joined by the large sub-unit along with a molecule of tRNA which has the anti-codon 'UAC' and which carries the amino acid methionine. As the three different RNA types come together, the interface between the small and large sub-unit of the ribosome forms two binding sites allowing two triplet codons be lined up side by side. The process of translation can then proceed.

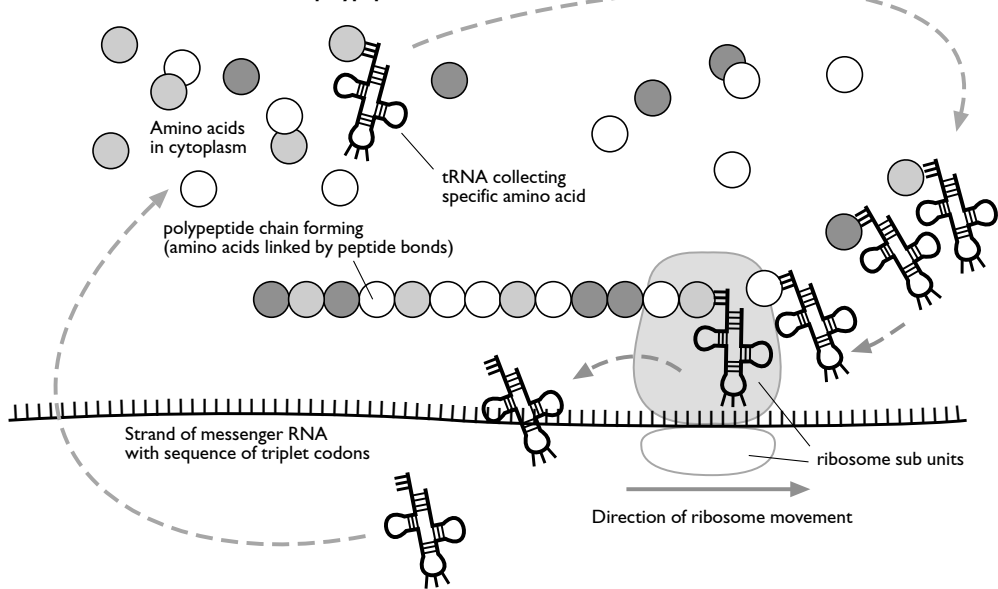
A second tRNA now occupies a position on the mRNA alongside the first and, as it does so, a peptide bond is formed between the two

amino acids. The ribosome moves along the length of the mRNA, and once a tRNA molecule has delivered the appropriate amino acid it is released to repeat the process. New tRNA molecules with their amino acids come to occupy the ribosomal coding sites, and the polypeptide chain grows ever longer. When the ribosome reaches the '**Stop**' **codon** (UAG) on the mRNA, it splits into its separate sub-units releasing the mRNA and the now complete polypeptide. A number of ribosomes move along the mRNA at the same time so that many polypeptide molecules are formed simultaneously. It is calculated that, in the average mammalian cell, more than a million new peptide bonds are being formed every second.

Generally mRNA molecules only have a short existence, sometimes just a few minutes, so that if continuous supplies of a particular protein are required the above processes must be repeated.

Transcription



Translation of mRNA code to build a polypeptide**◆ CHECKPOINT SUMMARY**

- ◆ The sequence of bases (and hence nucleotides) on one strand of the DNA molecule carries the genetic code for the synthesis of proteins and hence ultimately living cells.
- ◆ Three bases (a triplet codon) code for one amino acid.
- ◆ There are 64 possible combinations of any three from four bases, but only 20 amino acids to be coded for.
- ◆ Amino acids are coded for by more than one triplet codon and the code is said to be 'redundant' or 'degenerate'.
- ◆ A gene is a sequence of nucleotides of a DNA molecule which codes for a polypeptide.
- ◆ A gene for a polypeptide has a specific 'start' codon which thus sets the order of all the subsequent triplet codons
- ◆ Thus the code is non-overlapping i.e. a base can only be 'used' in one triplet codon.
- ◆ Only a fraction of the nuclear DNA contains genes coding for proteins, the rest consists of so-called 'junk' DNA the function of which is not understood
- ◆ Nuclear DNA with the genetic code never leaves the nucleus in eukaryotic cells.
- ◆ The genetic code for a polypeptide (a gene) is copied (transcribed) into mRNA which leaves the nucleus and associates with the ribosomes.
- ◆ The ribosomes 'read' the genetic message on the mRNA and tRNA deliver amino acids in the sequence determined by the code.
- ◆ There are 20 different types of tRNA, each specific to a particular amino acid.
- ◆ Each tRNA has a specific binding site at which the specific amino acid combines and an anti-codon which matches the specific codon for that particular amino acid.
- ◆ The amino acids combine by means of peptide bonds to give a polypeptide chain.
- ◆ The polypeptides subsequently form proteins.
- ◆ As enzymes are proteins their synthesis is controlled by DNA.

DNA control of enzyme synthesis

Since the base sequence of DNA in a gene controls the production of one specific polypeptide or protein, it follows that any change in this base sequence (a gene mutation) may lead to the production of a faulty protein. Even a substitution or deletion of a single base can alter the translation of the DNA code into a sequence of amino acids in a polypeptide chain. If the primary structure of a polypeptide is altered this can affect its secondary, tertiary and quaternary structures, with corresponding effects on its function. The presence of such a faulty protein may in turn affect the appearance and functioning (phenotype) of the organism as a whole and cause a genetic disease or disorder. Gene mutations which occur during the production of gametes are thus the cause of genetic diseases in the offspring.

Some genetic diseases are referred to as 'inborn errors of metabolism' because they affect one or more of the metabolic pathways involved in the normal functioning of the organism. They may, for example, include diseases where particular enzymes necessary for metabolic functions are faulty or absent, e.g. Phenylketonuria (PKU). In this example the pathway from faulty gene to abnormal phenotype is clear. Identification of the gene and protein product responsible for the disease can allow medical intervention at any point in the pathway from gene to disease.

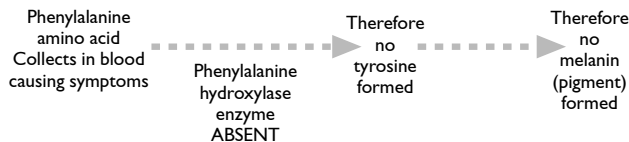
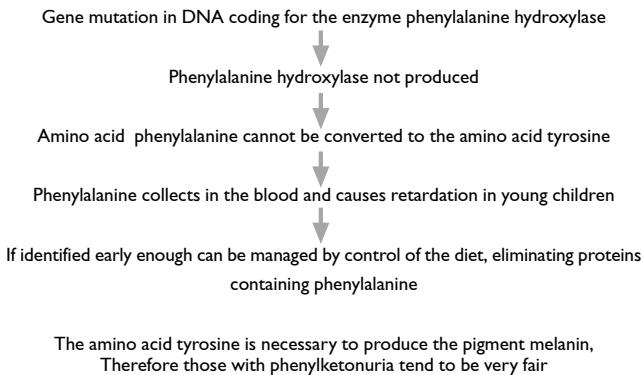
Phenylketonuria (PKU)

Most humans are able to convert some types of amino acids into other types of amino acid in the liver and thus produce all the proteins necessary for growth, repair and functioning of tissues. This explains why some amino acids are said to be essential (must be taken in the diet) whilst others are non-essential (do not need to be taken in the diet as they can be made from the essential amino acids in the body). Conversion of one amino acid to another (transamination) allows the body to make full use of ingested proteins and also prevents the build up of particular unused amino acids which could be dangerous.

In the disease phenylketonuria (PKU), however, a gene mutation in the DNA coding for the enzyme phenylalanine hydroxylase means that this enzyme is not produced in the liver. Without phenylalanine hydroxylase the body cannot convert the amino acid **phenylalanine** to the amino acid **tyrosine**. This results in an accumulation of phenylalanine in the body which severely affects many systems. Untreated sufferers may be both mentally and physically retarded.

PKU can be treated by modifying the diet to ensure that it lacks, or is very low in, the amino acid phenylalanine. The amino acid tyrosine may also be given. Under these circumstances individuals will not develop any signs of retardation. In the UK, all new-born babies are screened for PKU using a simple blood test which allows individuals with this genetic condition to be detected and treated before damage occurs. Treatment is only necessary in children, as adults seem to be unaffected by the high phenylalanine levels.

Phenylketonuria



SUMMARY OF PKU

- ▼ Gene mutation occurs in DNA coding for the enzyme phenylalanine hydroxylase
- ▼ Enzyme phenylalanine hydroxylase not produced
- ▼ Faulty metabolic pathway: amino acid phenylalanine not converted to tyrosine
- ▼ Phenylalanine accumulates with toxic effects
- ▼ Abnormal phenotype: Mental and physical retardation occurs

Cystic fibrosis

Cystic fibrosis is one of the most common autosomal recessive genetic diseases and affects about 1/2000 babies born in the UK. About 1:23 of the population are carriers of the cystic fibrosis allele. The disease which primarily affects the lungs, pancreas and liver is characterised by the production of thick, sticky mucus by epithelial cells in these regions. It usually causes death by early adulthood.

The disease is caused by a faulty protein known as **CFTR** (cystic fibrosis transmembrane regulator) which regulates transmembrane chloride ion channels in epithelial cells. A deletion of 3 bases in the DNA code of the CFTR gene means that one amino acid (phenylalanine) is missing from the CFTR protein which is produced. This change in the primary structure of the protein alters its secondary and tertiary structures, preventing it from responding to signals to open chloride channels in cell membranes. The overall effect of this is a build up of ions inside the cells which prevents water leaving by osmosis as it would do normally. The mucus released by cells is thicker than in normal individuals and sticks to cells causing inflammation and tissue damage, encouraging infections and preventing normal functioning of the organs concerned.

Although the disease is not fully understood, a number of treatments are available. There is, however, no cure. One experimental approach to the treatment of Cystic fibrosis in the lungs involves a form of gene therapy in which sufferers inhale normal CFTR genes incorporated into viruses or liposomes. Other treatments involve attempts to stimulate chloride ion secretion by the cells with the use of ATP.

SUMMARY OF CYSTIC FIBROSIS

- ▼ Deletion of bases occurs in DNA of CFTR gene
- ▼ Amino acid phenylalanine missing in CFTR protein
- ▼ Secondary and tertiary structure of CFTR disrupted
- ▼ CFTR protein fails to regulate opening of chloride ion channels in epithelial cells
- ▼ Chloride ions stay inside cells, as does water; and sodium ions are attracted in.
- ▼ Mucus produced is thick and sticky
- ▼ Mucus adheres to epithelial cells and causes disease symptoms

Cystic Fibrosis

Mutation causes the deletion of 3 bases in DNA so that one amino acid (phenylalanine) is not coded for in the CFTR protein (Cystic fibrosis transmembrane regulator)



Faulty CFTR protein cannot control the opening of chloride channels in the cell membrane



Results in production of thick sticky mucus, especially in lungs, pancreas and liver
Organs cannot function normally and infection becomes more likely

Treatment by Gene Therapy

CFTR gene identified on chromosome 7 in 1989

The normal gene for CFTR protein is inserted into a bacterial plasmid (DNA).
This is enclosed in a liposome (hollow lipid container)

The liposomes are introduced into the body of the sufferer using a nasal spray

The liposomes reach the lung epithelial cells and hopefully some of the normal CFTR genes are incorporated into the cells' DNA.
(The normal gene could also be introduced using a 'safe' virus)

◆ CHECKPOINT SUMMARY

- ◆ Some genes necessary for metabolic functions are faulty or absent, e.g. Phenylketonuria (PKU) and cystic fibrosis.
- ◆ In the disease phenylketonuria (PKU) a gene mutation in the DNA coding for the enzyme phenylalanine hydroxylase means that this enzyme is not produced in the liver.
- ◆ Without phenylalanine hydroxylase the body cannot convert the amino acid phenylalanine to the amino acid tyrosine.
- ◆ This results in an accumulation of phenylalanine in the body which severely affects many systems. Untreated sufferers may be both mentally and physically retarded.
- ◆ Cystic fibrosis primarily affects the lungs, pancreas and liver; and is characterised by the production of thick, sticky mucus by epithelial cells in these regions. It usually causes death by early adulthood.
- ◆ The disease is caused by a faulty protein known as CFTR (cystic fibrosis transmembrane regulator) which regulates transmembrane chloride ion channels in epithelial cells.
- ◆ A deletion of 3 bases in the DNA code of the CFTR gene means that one amino acid (phenylalanine) is missing from the CFTR protein which is produced.
- ◆ This change in the primary structure of the protein alters its secondary and tertiary structures.
- ◆ CFTR protein fails to regulate opening of chloride ion channels in epithelial cells
- ◆ Chloride ions stay inside cells, as does water. Sodium ions are attracted in.
- ◆ Mucus produced is thick and sticky
- ◆ Mucus adheres to epithelial cells and causes disease symptoms.

6

Gene technology

Contents

Recombinant DNA

- ▼ The production of recombinant DNA and its use in the production of human insulin and other proteins
 - isolation of the required gene
 - use of the enzymes: reverse transcriptase, restriction endonuclease and ligase
 - sticky ends: insertion of the gene into a vector and its subsequent introduction into host cells; plasmids and viruses as examples of vectors
 - use of genetic markers such as genes conferring antibiotic resistance to detect genetically modified organisms
 - multiplication of host cells

The moral and ethical issues associated with recombinant DNA technology

RECOMBINANT DNA

Imagine how simple life would be if there were only one type of computer and only one type of software. All the machines could then use the same language and all the information would be interchangeable. This is exactly how it is with the language of the genes. The synthesis of polypeptides from amino acids relies on exactly the same mRNA codons in every living organism so, for example, 'GCG' codes for the amino acid alanine and 'CGA' codes for arginine whether it occurs in a human, a honey bee or a mushroom. Now that genes can be identified and isolated, gene technology is providing a means for transferring them from one organism to another.

The terms 'genetic modification', 'genetic engineering' and 'gene technology' refer to the processes and techniques by which genes may be extracted from the DNA of one organism and inserted into the DNA of another organism (the **host** organism). In this way, the gene set (**genome**) of the host can be **transformed** (genetically modified). The modified DNA is called **recombinant DNA** because it has been re-combined to make a different set of codes.

Examples of genetically engineered products

Insulin is needed by people who are unable to produce sufficient quantities of the hormone and who would otherwise develop the disease **diabetes mellitus**. This is a condition, affecting 2% of the population, in which blood sugar rises to dangerous levels. Before gene technology, the only source of insulin was natural. It was extracted from the pancreases of pigs and cattle. This was not ideal because human insulin is slightly different in chemical structure,

and although the pig and cattle version works on glucose in a similar way, some people are allergic to it and the process is both costly and open to contamination by impurities.

In the early 1980s the gene for human insulin was transferred from human pancreas cells to *E. coli* bacteria. Bacterial colonies modified in this way were cultured for mass production in industrial fermenters providing a constant supply of insulin. Now most insulin is produced by transformed bakers' yeast cultures. Yeast has various advantages over bacteria. It does not produce any toxic by-products, it can be grown on simple nutrient media, and it can secrete the finished product directly into the culture medium making the harvesting process easier.

Factor VIII is a vital blood clotting factor lacking in people suffering from **haemophilia**. Natural sources are both expensive and liable to contamination but now the gene for factor VIII has been engineered into yeast cells.

The food industry uses more and more genetically engineered products. One of the first to be developed on a commercial scale was the enzyme **chymosin** (rennilase) which is used to coagulate milk for the production of cheese. Chymosin occurs naturally in the stomach of young mammals, where it is often referred to as rennin. The cheese making industry relied on extracts from calves' stomachs until the advent of gene technology. Now chymosin can be obtained from modified yeast cultures grown in fermenters.

The techniques of Genetic Manipulation

Gene technology relies on the fact that the genetic language is universal; the 'software' (genes) can be used in any living organism. The difficulty lies in the 'cutting and pasting' of genetic information from one organism to another, and you must first become familiar with three enzymes which are crucial to this process.

Reverse Transcriptase

Reverse transcriptase is an enzyme which makes a single strand of DNA from an RNA template. From this strand of DNA, a complementary strand can be assembled with the aid of DNA polymerase. Reverse transcriptase enzyme was identified and isolated from **retroviruses**, a group of viruses which includes the HIV virus. The genetic material of retroviruses is RNA (not DNA), and when retroviruses invade cells their RNA uses reverse transcriptase to make a piece of DNA. This becomes incorporated into the host cell's own DNA and directs the production of new viruses (an example of natural genetic modification of the host cell).

Reverse transcriptase is used in gene technology to make DNA from mRNA. Remember that mRNA is a copy of a single gene and that mRNA of a working gene may be found in the cytoplasm of a cell. It is possible to isolate a gene in the form of mRNA and then to multiply it using a combination of reverse transcriptase and DNA polymerase.

Restriction endonuclease enzymes

Another vital tool for gene technologists are enzymes called restriction endonucleases (restriction enzymes) which act like

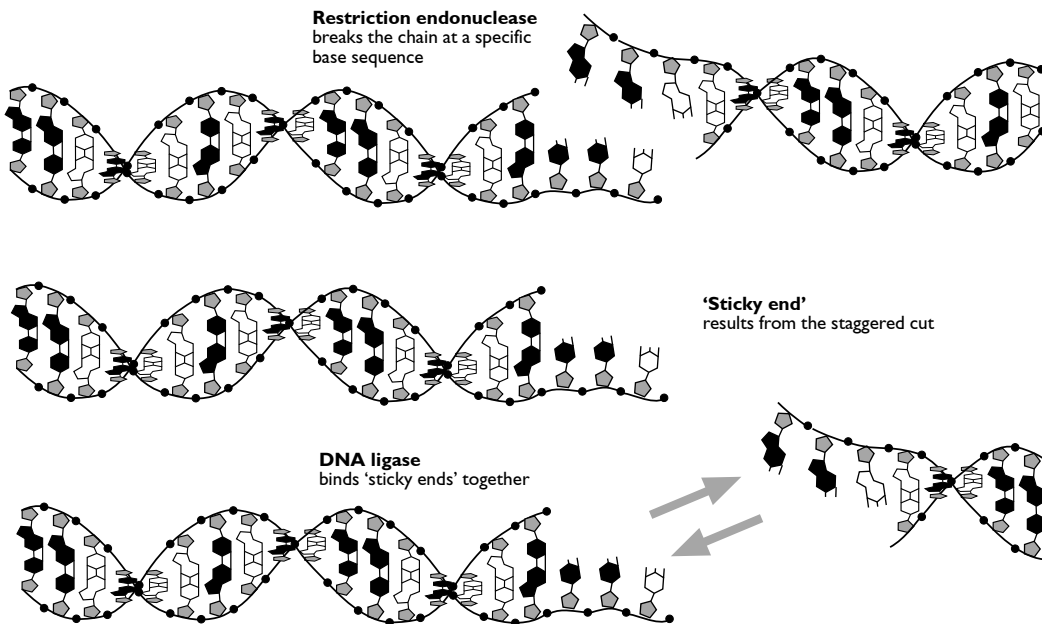
scissors to chop up DNA at specific base code sites into smaller polynucleotide fragments. Restriction enzymes are extracted from bacteria. In bacteria they serve to protect the organism from invading viral DNA. In gene technology they are used to cut DNA into fragments which can be separated and isolated. Often the cut leaves 'sticky ends' which are exploited in another stage of the process.

DNA ligase

To 'ligate' is to join. DNA ligase is a joining enzyme. It is used to stick the sticky ends of DNA fragments together creating new combinations (**recombinant DNA**).

The techniques for transferring genes from one organism to another can be considered in five main stages.

Action of restriction and ligase enzymes



Isolation of the required gene

It is surprisingly easy to extract DNA from living cells as you may already have discovered in the laboratory. Using restriction enzymes and gel electrophoresis it is possible to separate and isolate different DNA fragments, even pure genes. Alternatively, genes can be extracted from cells in the form of mRNA as described above and converted into DNA using reverse transcriptase.

The use of a vector

A vector is a carrier. The most common vectors in gene technology are bacterial plasmids and

bacteriophage viruses, both of which can carry the required gene to the host cell.

Plasmids are rings of DNA found in bacterial cells which are not part of the main bacterial chromosome. They are easily transferred from one bacterial cell to another. Plasmids can be cut at specific sites using restriction enzymes to leave sticky ends. If the same enzyme is used on the isolated gene then the sticky ends will match and the two can be joined using DNA ligase, so that the plasmid contains the recombinant DNA.

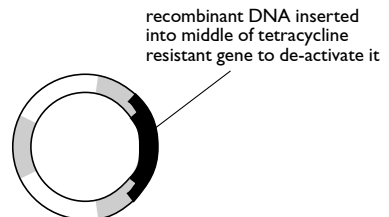
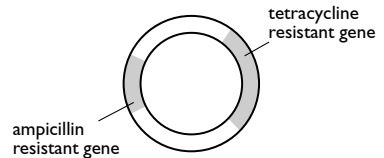
Bacteriophages are viruses which infect bacteria. They have a simple ring of DNA which they inject into the bacterial cell. This ring of DNA resembles a plasmid and can be modified in the same way to contain the required gene.

Transformation of the host cells

The vector is now brought into contact with the host cells, in the hope that some of the recombinant DNA might be taken up by the host cells. In order to discover whether or not the host cells have been transformed, **marker genes** (selection genes) are used. These are genes which are engineered into the vector alongside the required gene. They do not harm the host cell in any way but code for products which show up in an easily identifiable form in the host cell, confirming that the process of transformation is complete. One of the more common marker genes codes for antibiotic resistance. The transformed host cells can be isolated by introducing an antibiotic to the culture. All non-transformed cells are killed by the antibiotic, but the transformed cells survive as a result of the antibiotic resistant marker gene.

There has been some concern expressed about the use of antibiotic resistant genes. Opponents of this technique point to the risk of such genes escaping and entering disease organisms. Imagine the potential of a disease-causing bacterium, genetically modified to resist antibiotic treatment.

Inserting recombinant DNA

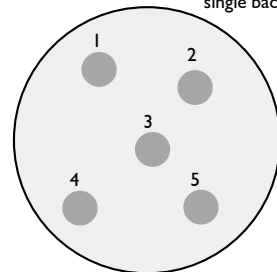


To select the bacteria with the recombinant DNA plasmid colonies are grown from individual bacteria on sterile agar plates.

Replica plating

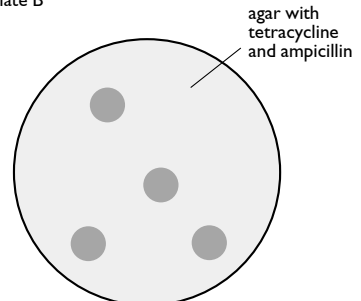
Plate A

Five colonies, each grown from single bacteria



A sterile plate is pressed upon plate A and then pressed upon the sterile agar of plate B. This leaves plate A intact and passes the identical pattern of bacterial growth onto plate B.

Plate B



Colony 2 has failed to develop, these bacteria must have the de-activated tetracycline resistant plasmid. These bacteria can be cloned and cultured from the colony growing on plate A.

Multiplication of transformed cells

Once a cell or group of cells has taken up recombinant DNA, the next step is to culture the cells in a suitable growth medium, allowing them to grow and multiply. In the case of transformed bacterial and yeast cells, this can be done on a large scale in industrial fermenters, allowing the product(s) of the new gene to be harvested and purified, as in the case of insulin, cofactor VIII and chymosin.

QUESTIONS RAISED by GENE TECHNOLOGY

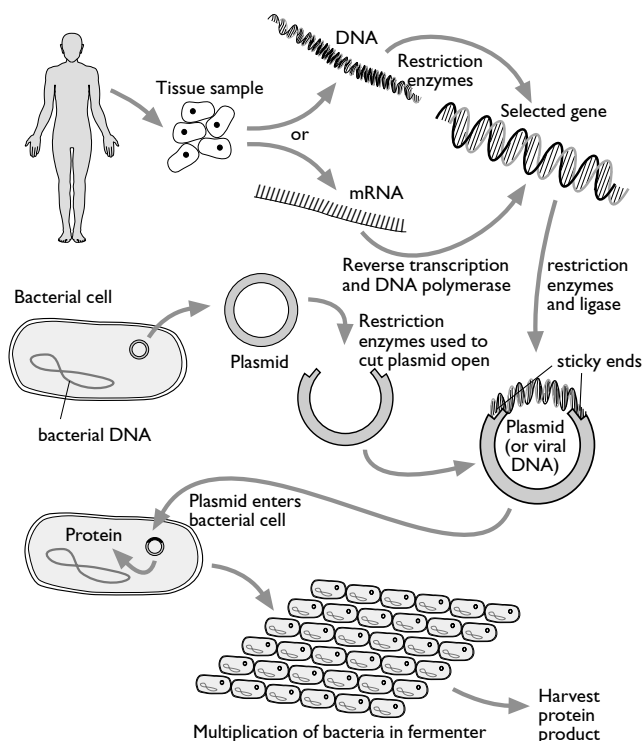
Gene technology raises many hopes for the future. It should be possible for example to expand the tolerance range and extend the habitat of many crop plants by engineering resistance to salinity, heat, cold, and wind. New varieties will compete better with weeds and be more tolerant of attack from insects and fungal disease. Nitrogen fixation will be enhanced, and renewable energy crops will begin to make a realistic difference to the greenhouse gas levels. Malnutrition may be overcome by staple food crops with a complete complement of essential amino acids, and new drugs will be developed from plant substances.

It also raises a number of questions about the way we want to live. Genetic modification has brought in the age of the designer plant, potatoes exclusively designed for oven chips, square tomatoes, straight bananas, salad vegetables, and fruits of every colour and texture. If there is a market for it, then the plant scientists will be able to engineer it. However, the accidental transfer of new genes into wild organisms could lead to herbicide resistant weeds and antibiotic resistant bacteria. Transgenic plants can also lead to transgenic pests and microbes.

The world population, currently about 5.5 billion, is expected to double to 11 billion by the year 2050. Given that 1 billion are already classified by international agencies as 'poor' and 500 million live in 'absolute poverty', it is clear that food production must be more than doubled in the next 50 years, and this must be achieved on a reducing area of fertile land.

Plant biotechnology, already moving at a rapid pace, offers some hope for the future. Plant biotechnology however, is largely the property of 'First World' independent companies, and 97% of the world's population increase will occur in the developing nations. Furthermore, environmental safety procedures, which evolve with the industry, exist in, and apply only to the countries which have the technology, yet transgenic products now have world wide distribution. A great deal of international cooperation, not to mention finance, will be needed to overcome the problem of safely transferring the products of the new technology to the countries which need them most. The human genome project which is analysing the sequence of bases in human DNA, also raises many problems to be dealt with. Certainly some inherited conditions and tendencies will be better understood, and various treatments developed. On the other hand the potential for abuse is enormous, including refusal of insurance cover to certain groups, employment vetting, immigration control and many others.

Genetic engineering



◆ CHECKPOINT SUMMARY

- ◆ Isolation of the gene to be manipulated is the first step
- ◆ It is easier to find and isolate the appropriate mRNA which has been transcribed (copied) from the relevant DNA, than it is to try and isolate the particular length of DNA itself.
- ◆ The enzyme reverse transcriptase can then be used to make a copy of the DNA from the mRNA.
- ◆ Plasmids (circular DNA) are isolated from bacteria.
- ◆ Plasmids are cut open by restriction enzymes and the length of DNA is inserted and the plasmid resealed by ligase enzymes.
- ◆ The genetically modified plasmids are then reintroduced into the bacteria.
- ◆ The bacteria multiply and produce the protein coded for by the inserted DNA.
- ◆ To identify those bacteria which have successfully taken up the modified plasmids marker genes are incorporated along with the required gene.
- ◆ Marker genes include those for antibiotic resistance so that the culture can be flooded with antibiotic and only the bacteria with the marker gene will survive.
- ◆ Two early successes with these techniques were the synthesis of human insulin for the treatment of diabetics, and blood clotting factor VIII for the treatment of haemophiliacs.

7

Non-communicable disease

Contents

The biological basis of heart disease

- ▼ Atheroma as the presence of fatty materials within the walls of arteries
- ▼ Explanation of the link between atheroma and the increased risk of aneurysm and thrombosis
- ▼ Myocardial infarction and its cause in terms of an interruption to the blood flow to the heart muscle
- ▼ Risk factors associated with coronary heart disease
- ▼ Blood cholesterol, cigarette smoking and increased blood pressure.

The biological basis of cancer

- ▼ The main characteristics of tumours and tumour cells
- ▼ The distinction between benign and malignant tumours
- ▼ Consideration should be given to the following aspects:
 - genes and the part they play in the control of normal cell growth
 - chemical carcinogens and radiation may damage DNA and cause mutations in the genes controlling growth
 - tumour cells failure to respond to normal growth regulating processes; metastasis and the invasion of other organs
 - the role of tumour suppressor genes in preventing tumour growth
- ▼ The incidence of lung and skin cancers in the United Kingdom. Factors which increase the incidence of these cancers. Interpretation of data showing the pattern of incidence and links with possible causal factors
- ▼ The moral and ethical issues associated with cigarette smoking and disease.

Introduction

The term 'non-communicable' or non-infectious disease refers to a broad category of diseases which includes all those conditions not caused by pathogens. It includes genetic diseases, mental diseases, self-inflicted diseases and the multifactorial degenerative diseases which include cancers and cardiovascular diseases.

Diseases in this latter sub-group have rapidly become the largest and most significant cause of illness and death across the Developed World during the twentieth century. Deaths from such diseases are also increasing rapidly in many developing countries, and in some have overtaken infectious diseases as leading causes of death. Whilst degenerative diseases mainly affect people in middle age or above, many of the diseases including cardiovascular disease, some cancers, diabetes, and obesity are the result of a life time of exposure to a range of risk factors.

The BIOLOGICAL BASIS of HEART DISEASE

Cardiovascular Disease

The cardiovascular system includes the heart and the blood vessels through which blood is pumped around the body. Cardiovascular disease is thus a term which covers several different diseases within this system, including **high blood pressure** (hypertension), **atherosclerosis** (narrowing of the arteries with fatty deposits) and **coronary heart disease** (atherosclerosis specifically affecting the coronary arteries which supply the heart). This latter disease may lead to fatal heart attack or **myocardial infarction**. This is the cause of death in 110,000 people in England and Wales each year.

Atherosclerosis

Atherosclerosis is a condition in which the large arteries of the body (especially the aorta, carotid arteries and coronary arteries) become hardened and narrowed by the build up of deposits inside the artery wall. These deposits, which are known as atheromata (plural of atheroma) are composed of cholesterol and other fatty materials, which come from the blood plasma. Whilst atherosclerosis is usually only found in adults, atheroma deposits may start to build up from childhood.

Although not fully understood, atheroma build up is thought to begin in areas of the arteries which have been damaged. Damage to the inner lining or endothelium, perhaps caused by high blood pressure (overstretching of the artery walls), or by the effect of chemicals derived from cigarette smoking, allows lipids (especially cholesterol) in the plasma to enter the artery wall. The lipids accumulate beneath the smooth muscle layer, appearing at first as small fatty streaks which later become fibrous and hardened as connective tissue builds up around the site. The region becomes known as a plaque. The artery wall will thus start to bulge into the lumen. The plaque may also damage the elastic and muscle tissues in the artery wall.

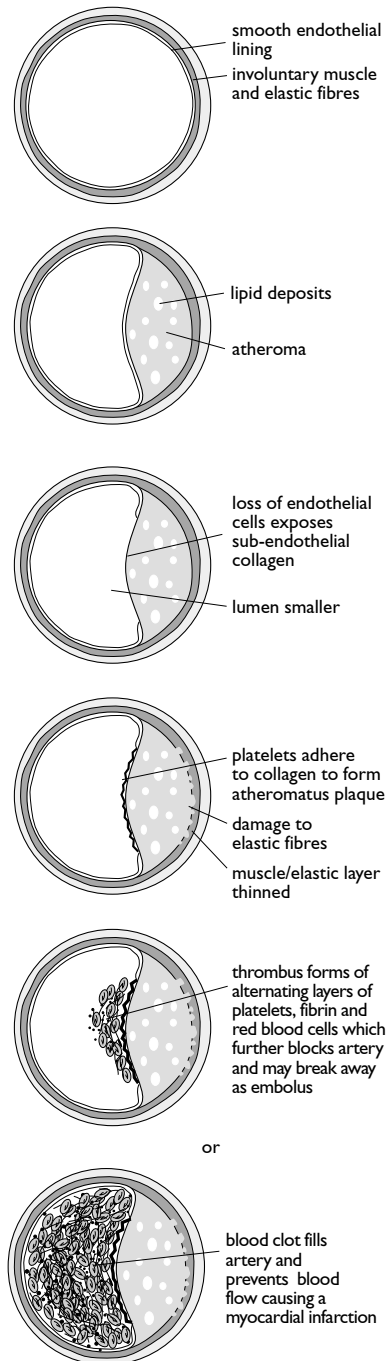
The effects of atherosclerosis: high blood pressure, aneurysm and thrombosis.

Atherosclerosis is a serious risk factor in many other cardiovascular conditions. Firstly it may lead to high blood pressure which is linked to the loss of elasticity in the arteries. Resistance to blood flow is increased, forcing the heart to pump blood at a higher pressure to reach all the tissues.

As discussed above, atherosclerosis may weaken the artery wall since the fibrous plaque disrupts the normal structure of the wall. In particular the layers of muscle and elastic tissue (the tunica media) may become thinner. In some cases this can lead to an expansion of the artery known as an aneurysm (a balloon like swelling of the vessel). This may burst, and since the artery most affected by aneurysms is the aorta, this is usually fatal if immediate surgical help is not available. High blood pressure may be a contributing factor in the development of aneurysms.

A further complication of atherosclerosis is that of thromboses or internal blood clots. In diseased arteries, the roughened endothelium covering a plaque may rupture, allowing blood to flow into the

Thrombosis Development in Coronary artery



plaque. Platelets from the blood are attracted to the damaged area and stick to the artery wall where they release clotting chemicals. Gradually a thrombus will form. Similar mechanisms to those involved in blood clotting at a wound site are involved (see section 12.3) Once formed the thrombus may cause damage in one of two ways. It may completely block the artery preventing blood flow to areas of tissue. If this occurs in a coronary artery this can lead to myocardial infarction, which occurs when parts of the heart muscle are starved of oxygen and glucose. Without oxygen and glucose the muscle tissue cannot respire and thus has no ATP for contraction of the muscle fibres. The heart then fails to contract properly and death may follow.

Myocardial infarction may occur suddenly without prior warning or may be preceded by pain in the chest region (angina), this pain is caused by narrowing of the coronary arteries and their inability to supply adequate oxygen to heart muscle especially during exercise.

In other cases the clot may detach itself from the artery wall and enter the circulation. Known as an **embolus** this clot may then block a vessel in another part of the body. Often they lodge in the pulmonary artery, or in vessels of the brain leading to a stroke. Such blockages of vessels can lead to death, or tissue damage, but in other cases may be treated by giving drugs to break down the clot, e.g. warfarin or heparin which reduce blood clotting.

Risk Factors in coronary heart disease

In common with other non-communicable diseases, coronary heart disease (CHD) is not caused by one factor but has a number of 'risk factors' associated with it. Each risk factor increases the risk of developing coronary heart disease, although none of them are sufficient to bring about the disease on their own. In some cases the link between the factor and the risk of CHD is not well understood. Risk of CHD may build up by long exposure to known risk factors many of which are avoidable.

The following have been identified as major risk factors:

High blood cholesterol level. There is a strong positive association between high levels of cholesterol and other saturated fats in the blood and risk of CHD. Such lipids do not exist free in the blood but in compounds of lipid and protein known as Low Density Lipoproteins (LDL). Such high levels of LDLs have dietary causes (diets high in saturated fats such as are found in butter and some meats), and genetic causes, by which some people will naturally have high levels of LDL even if their diet is low in cholesterol.

Interestingly the consumption of some polyunsaturated fats which travel in the blood as high density lipoproteins seem to have a protective effect on CHD. It is thought that these HDLs reduce plasma levels of LDLs perhaps through altering the metabolism of cholesterol in the body. Polyunsaturated fats are found in many vegetable oils, including olive oil. The relative proportions of HDLs and LDLs are important.

Cigarette smoking. Smoking is thought to be responsible for up to 40% of all deaths from CHD. A person who smokes 25 cigarettes per day is up to 15 times more likely to develop CHD than a non-smoker. The reasons for this are still not well understood, but the following effects have been noted:

Carbon monoxide (CO) found in tobacco smoke combines irreversibly with haemoglobin in red blood cells producing carboxyhaemoglobin. This compound cannot carry oxygen so oxygen delivery to tissues may be reduced. This may in turn cause heart rate and blood pressure to increase. Also, carbon monoxide, along with nicotine, is toxic to the endothelial lining of arteries and is thought to be one factor which may cause damage to this lining and allow cholesterol to enter the vessel wall causing development of atheroma.

Nicotine, the addictive component of cigarette smoke has a number of effects on the cardiovascular system. It causes the blood vessels to constrict, increasing blood pressure and heart rate. This puts extra strain on the heart and can be serious for the person who has already had a coronary attack. Nicotine also increases the concentration of fatty acids in the blood, which may contribute to the deposition of atheroma. This along with the fact that nicotine makes blood platelets more sticky, increases the possibility of blood clotting inside blood vessels leading to thrombosis. At the same time more fibrinogen (one of the proteins involved in blood clotting) is produced, while lower levels of enzymes which break down blood clots are found. Blood clots formed in the coronary arteries may block the artery leading to a myocardial infarction or heart attack. Nicotine also makes the heart more irritable and can precipitate dangerous irregularities in heart beat.

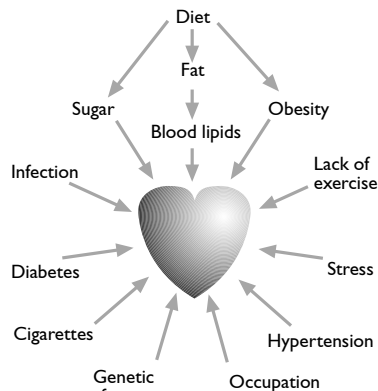
High blood pressure is closely linked to many other risk factors in CHD including smoking, obesity, diabetes, stress, high salt intake and lack of exercise. It is thus difficult to consider the effects of high blood pressure in isolation.

Lack of exercise is often considered to be one of the most important and most easily corrected risk factors in CHD. Exercise in itself can increase the strength of the heart muscle and decrease the resistance of blood flow in vessels. It also helps to prevent obesity, reduce stress and is often associated with diets lower in saturated fats.

Age and sex. Whilst CHD can affect young people it is relatively rare in those younger than about 40 years. It must be stressed, however, that accumulation of atheroma may begin in childhood, but its effects in terms of CHD may not be seen for several decades. CHD is much more common in men than women in middle age. This effect decreases in post-menopausal women and a protective effect of oestrogen has been suggested.

It should be noted that with the exception of age, sex and genetic predisposition, all of the major risk factors for CHD can be avoided.

Heart disease factors



◆ CHECKPOINT SUMMARY

- ◆ Atheroma (pleural atheromata) are fatty deposits of cholesterol and other fatty materials in the walls of large arteries (especially the aorta, carotid arteries and coronary arteries) leading to the condition atherosclerosis.
- ◆ Lipids accumulate beneath the smooth muscle layer, appearing at first as small fatty streaks which later become fibrous and hardened as connective tissue builds up around the site. The region becomes known as a plaque.
- ◆ Atherosclerosis may lead to high blood pressure which is linked to the loss of elasticity in the arteries, and can lead to an expansion of the artery known as an aneurysm, and thromboses or blood clots which may occur in any diseased artery.
- ◆ Once formed the thrombus may completely block a coronary artery this can lead to myocardial infarction, which occurs when parts of the heart muscle are starved of oxygen and glucose.
- ◆ In other cases the clot may detach itself from the artery wall and enter the circulation. Known as an embolus this clot may then block a vessel in another part of the body. Often they lodge in the pulmonary artery, or in vessels of the brain leading to a stroke.
- ◆ Risk factors associated with coronary heart disease include, high blood cholesterol level, cigarette smoking and its associated carbon monoxide (CO) and nicotine, high blood pressure, lack of exercise, and is much more common in middle aged men.

The BIOLOGICAL BASIS of CANCER

Introduction

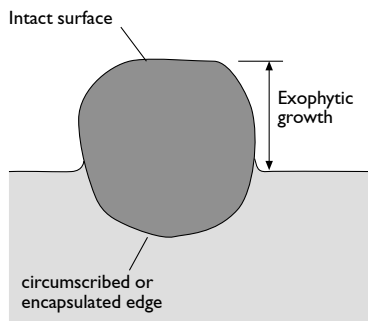
In 1999 7.3 million people worldwide died from cancers, of whom 1 million were in high income European countries. In such countries cancers now rank as the second most important cause of death (behind cardiovascular disease), with by far the most common being lung cancer. Other cancers which cause large numbers of deaths are prostate cancer in men, breast cancer in women and cancer of the colon in both sexes. Cancer is often classified as a degenerative disease, so it is not surprising that the vast majority of these deaths occur in older adults. It should be noted that cancer is not a single disease but rather a complex group of several hundred different diseases which share common characteristics. Chief amongst these is the presence of unregulated cell growth leading to the development of tumours.

The main characteristics of tumours and tumour cells.

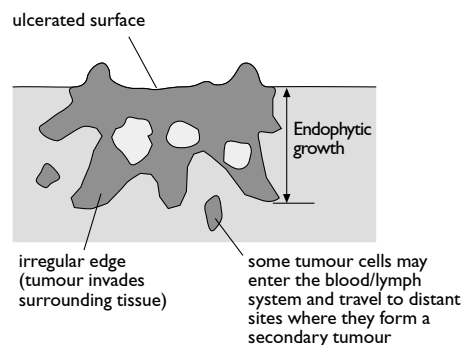
A tumour is an abnormal mass of tissue, the growth of which is uncoordinated and exceeds that of normal tissue. Tumours are divided into **benign** and **malignant**. The principal difference is that malignant tumours invade their surrounding tissues and spread to distant sites via the blood stream to form secondary tumours, known as **metastases**; while benign tumours usually grow at a slower rate, they never invade organs or blood vessels/lymphatics nor do they metastasize. Such tumours are more easily removed during surgery than malignant ones.

Tumour cells, whether benign or malignant, differ from normal cells both in behaviour and appearance.

Benign tumour



Malignant tumour



Differences in behaviour of tumour cells

Tumour cells often grow rapidly and out of control. When normal cells are grown in culture, for example on an agar plate, they will divide and spread out over the surface of the plate. Once they have covered the plate in a layer one cell thick, cell division stops. In contrast, malignant cells grown under the same conditions continue to grow in a heaped up multilayered form.

Differences in appearance of tumour cells

Although tumour cells may arise in many different sites in the body, they have the following differences to normal cells:

- ▼ wide variation in the shape and size of the cell
- ▼ large nucleus when compared to the size of the cell, which stains darkly.

As a general rule benign tumour cells resemble the normal cell type from which they have arisen, while malignant tumour cells do not.

Classification of tumour cells

Tumours are classified firstly as benign or malignant, and secondly by their organ of origin, such as the breast, or even by their original cell type.

Identification is usually made on the basis of a biopsy. This is a representative fragment of the tumour removed and examined by a pathologist under the microscope. Once the type of tumour has been identified, the likely course of the disease (prognosis) and treatment can be determined.

Causes of cancer

Chemical carcinogens and radiation may damage DNA and cause mutations in the genes controlling growth.

Chemical carcinogens

A chemical that increases the risk of developing a cancer is referred to as a **carcinogen**, or as being carcinogenic. These include chemicals found in the tar of cigarette smoke, in car exhaust fumes, and in low levels in some common foods and food additives.

Radiation

Ultra-violet light, gamma rays and X-rays are also carcinogenic. Radiation damages DNA and malignant transformation is more likely.

The Role of Genes in Normal Cell Growth and Cancer

The normal growth of cells is regulated by growth factors. These are generally polypeptides present in the blood plasma, that bind to receptors on the cell surface. This **growth factor-receptor complex** then activates an enzyme which in turn will activate second messengers. These second messengers stimulate factors that start DNA transcription, and the synthesis of proteins necessary for cell growth and division.

Cancer is a term used to describe a huge range of diseases. However, at the molecular level they all share common features. The uncontrolled growth which exceeds that of normal tissue, results from mutations in the genes that encode for proteins regulating the normal growth of cells and the cell cycle.

The majority of mutations in the genome have no influence on cell growth. Indeed some mutations result directly in the death of the cell. However, some gene mutations result in the cell escaping from the normal controls that regulate their growth and division. Significant mutations are rare events and often several mutations in a single gene are required before a tumour develops. However, one single transformed cell can give rise to a tumour through repeated cell division. A tumour is therefore usually monoclonal: all the cells are derived from a single mutated precursor cell.

Tumour suppressor genes encode for proteins which suppress cell growth in the normal cell. It is thought that many of these genes encode proteins which suppress DNA synthesis. Thus a mutation in one of these suppressor genes may result in production of a defective protein and loss of suppression of cell growth and division, resulting in the development of a tumour.

Programmed cell death (apoptosis) is part of the normal cell cycle and involves many proteins including enzymes. Disruption of the genes encoding these proteins may cause cells to escape programmed cell death and continue to divide to form a tumour.

Lung Cancer: incidence and causes.

Lung cancer accounts for 19% of all cancers and 27% of cancer deaths in Britain, and causes an estimated 1.2 million deaths worldwide in 1999. It is the most common cancer in British men, although the incidence is no longer rising. Whilst breast cancer remains the most common cancer in women, the incidence of lung cancer in this group is rising. The reasons for this are discussed below. Overall, lung cancer causes over 40 000 deaths per year in the United Kingdom.

There is indisputable evidence that most lung cancer is caused by smoking; there is also an association with radiation exposure, asbestos and air pollution; and the evidence that passive smoking causes cancer is growing.

The link between smoking and lung cancer has been established by the work of Sir Richard Doll. He demonstrated the strong links between smoking and lung cancer, chronic bronchitis, and emphysema in a study which began in the 1950s. He used a technique known as the **prospective study** in which he identified his study group first, and then recorded the deaths from smoking-related diseases which occurred in the group over a number of years.

Smoking among secondary school children 1996

Increases shown in bold.

Boys	1982	1996
Age 11	1%	1%
Age 12	2%	2%
Age 13	8%	8%
Age 14	18%	13%
Age 15	24%	28%
Total	11%	11%

Girls	1982	1996
Age 11	0%	0%
Age 12	1%	4%
Age 13	6%	11%
Age 14	14%	24%
Age 15	25%	33%
Total	11%	15%

His study population were male general practitioners (GPs) who were happy to be involved in a medical trial, were of broadly similar socio-economic groups, had similar lifestyles, occupation, and exposure to other risk factors. He recorded the number of cigarettes smoked per day in this group and over a period of many years recorded deaths from lung cancer, bronchitis and emphysema.

He found that heavy smokers (more than 25 cigarettes per day) had a risk of lung cancer 25 times higher than non-smokers, and 10 cigarettes per day increased the risk 10-fold over that of non-smokers. Pipe smokers were at a lower risk, presumably because of lower amounts of inhalation. Risk of lung cancer in ex-smokers declined, so that 13 years after quitting smoking, the risk was the same as that of a non-smoker. Other factors (such as exposure to pollutants) were considered before the data was analysed.

Atypical cells may be seen in smoker's lungs, and in lung tissue close to tumours. These are probably a reflection of damage to DNA caused by smoking resulting in mutations. If the person continues to smoke, further genetic changes will occur and they will be at high risk of developing cancer.

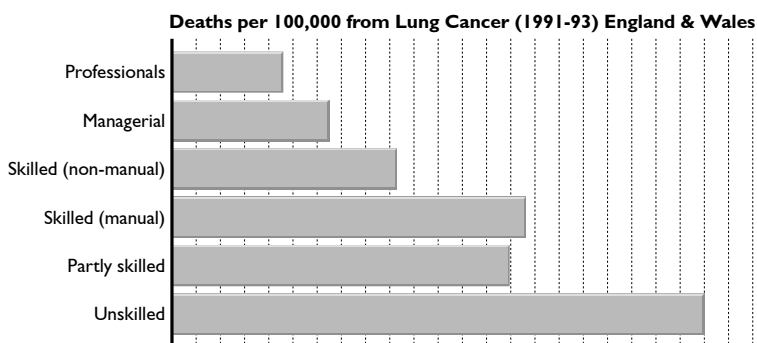
Skin cancer: incidence and causes

There are three main types of skin cancer, defined by the cell type from which they arise. Two types: **basal cell carcinoma** and **squamous cell carcinoma** are both very common. The main factor increasing their incidence is exposure to sunlight. It is thought that ultraviolet light in the sun's rays causes direct damage to DNA.

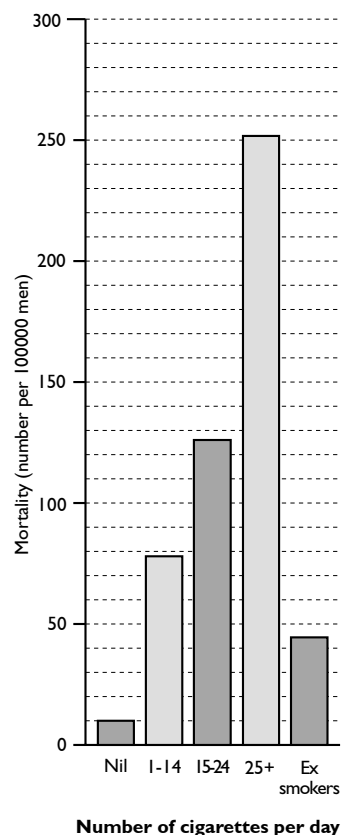
Several features of these two cancers implicate sunlight as an important cause.

- ▼ They most commonly occur in elderly patients, with a lifetime of exposure to the sun (and thus the chance to accumulate mutations in their DNA).
- ▼ The tumours are most commonly found on sun exposed areas of the body, e.g. the face and arms.
- ▼ They are more common in light skinned people, who have less melanin, allowing more ultraviolet light to reach and damage skin cells.

Deaths from lung cancer according to socio-economic group



Graph to show death rates from lung cancer according to smoking habits



- ▼ These cancers are more common in individuals who work outside, than in those who have office jobs.
- ▼ These cancers are more common in patients who undergo ultraviolet light therapy for treatment of other conditions, such as psoriasis.

The third type of cancer is malignant melanoma. Although rare, with 6 new cases per 100 000 per year in the UK, it is still the most common cancer in the 20-39 age group. Large epidemiological studies similar to those outlined for smoking and lung cancer, have established that sunlight exposure is strongly associated with malignant melanoma. Fair skinned individuals living close to the equator are most at risk. Thus while the incidence of melanoma in Black populations is only around 0.6 per 100 000 per year, it is as high as 33 per 100 000 per year in those of European descent living in Queensland, Australia.

The incidence of melanoma in the UK is rising at an alarming rate, and this almost certainly reflects changing attitudes towards sun exposure. Travel abroad is becoming more affordable, and many people strive hard for a tan, in the mistaken belief that it denotes healthiness. In contrast to the two other types of skin cancer outlined above where total sun exposure appears to be the main risk factor, in the case of melanoma, it appears that the risk is increased by episodes of severe burning.

◆ CHECKPOINT SUMMARY

- ◆ Cancer is not a single disease but rather a complex group of several hundred different diseases which share common characteristics. Chief amongst these is the presence of unregulated cell growth leading to the development of tumours.
- ◆ A tumour is an abnormal mass of tissue, the growth of which is uncoordinated and exceeds that of normal tissue.
- ◆ Tumours are divided into benign and malignant.
- ◆ Malignant tumours invade their surrounding tissues and spread to distant sites via the blood stream, the lymphatics or across body cavities to form secondary tumours, known as metastases
- ◆ Benign tumours usually grow at a slower rate, they never invade organs or blood vessels/lymphatics nor do they metastasize.
- ◆ Tumour cells have the following differences to normal cells: wide variation in the shape and size of the cell large nucleus when compared to the size of the cell, which stains darkly.
- ◆ As a general rule benign tumour cells resemble the normal cell type from which they have arisen, while malignant tumour cells do not.
- ◆ Causes of cancer include chemical carcinogens and radiation which may damage DNA and cause mutations in the genes controlling growth.
- ◆ Carcinogens are chemicals that increases the risk of developing a cancer; and include chemicals found in the tar of cigarette smoke, in car exhaust fumes, and in low levels in some common foods and food additives.
- ◆ Ultra violet light, gamma rays and X-rays are also carcinogenic. It damages DNA and malignant transformation is more likely.
- ◆ The uncontrolled growth seen in cancer; results from mutations in the genes that encode for proteins regulating the normal growth of cells and the cell cycle.
- ◆ A mutation in suppressor genes which code for proteins which suppress cell growth in the normal cell may result in a tumour.
- ◆ Mutations in genes which encode for proteins which control programmed cell death may cause cells to continue to divide to form a tumour.
- ◆ Lung cancer accounts for 19% of all cancers and 27% of cancer deaths in Britain and causes an estimated 1.2 million deaths worldwide in 1999.
- ◆ Skin cancer is correlated with radiation damage by UV light in sunlight, the incidence of melanoma in Black populations is only around 0.6 per 100 000 per year; but as high as 33 per 100 000 per year in those of European descent living in Queensland, Australia.

The moral and ethical issues associated with cigarette smoking and disease

This is a highly complex and controversial topic. The issues can most simply be divided into three areas: the impact of smoking on society, the individual smoker and the individual non-smoker.

Smoking is an contributing factor in around 50% of all hospital admissions. Apart from lung cancer, there is strong evidence linking smoking to bladder, laryngeal, mouth, oesophageal, stomach cancers and many other tumours. Bronchitis, emphysema, heart disease and peripheral vascular disease are much more common in smokers than non-smokers. Many people feel that smoking is a self inflicted disease, and therefore not a burden that the NHS and society should carry the cost of.

Others will argue that smokers have the same rights to the NHS as non-smokers. Many will argue that they started smoking before the risks of smoking were known, and they are now addicted and unable to give up. They would also claim that obesity and alcoholism are similarly self inflicted but few suggest that these groups should suffer discrimination. There is also truth in the claim that the heavy taxation on cigarettes contributes to the National Health Service (NHS). A lifetime of smoking (and increased taxation) would more than cover the extra burden of this group on the NHS. Also, the decreased life expectancy of smokers means that fewer live to retirement age when they can claim a pension from the State.

On an individual level, smokers argue that smoking is a legal pastime, and every individual has a right to smoke. However, there is emerging evidence that breathing in other people's smoke (passive smoking) increases a non-smokers risk of smoking related diseases. Non-smokers have used this to push through legislation restricting smoking in many public places.

Perhaps it is more generally agreed by society that individuals should be made fully aware of the risks of smoking, and those who wish to give up should be helped to do so. Despite health education programmes, and the warnings which now appear on all cigarette packets and advertising material, smoking remains widespread. The British Government has set targets for a 30% reduction in smoking related diseases in men and a 15% reduction in women by 2010. It is essential that such programmes target young smokers since the earlier a person starts smoking, the greater is their risk of death from related illnesses.

8

Disease may be diagnosed by a variety of techniques

Contents

DNA probes and diagnosis

- ▼ The use of DNA probes to identify the presence of specific genes associated with human disease
- ▼ This process in such detail as to show that:
 - restriction enzymes are used to cut DNA into fragments
 - electrophoresis is used to sort fragments according to charge and size
 - radioactive DNA probes are used to locate specific DNA fragments.

Enzymes and diagnosis

- ▼ Disease and changes in the concentration and distribution of enzymes in the body
- ▼ Pancreatitis and the increased concentrations of digestive enzymes in the blood or a decrease in the concentration of these enzymes in the gut
- ▼ The high sensitivity and specificity of enzymes allowing their use as analytical reagents. The use of glucose oxidase and peroxidase in testing for glucose.

Introduction

As discussed previously, diseases have a wide range of causes. Some, such as malaria and schistosomiasis are caused by pathogens; others, such as cystic fibrosis and PKU are caused by faulty genes; yet others, including CHD, cancers and diabetes result from a range of interacting environmental and genetic factors. Whatever the cause of the disease, correct diagnosis is essential if the disease is to be treated effectively.

The USE of DNA PROBES to IDENTIFY the presence of specific genes associated with human disease

Genetic screening is the name given to a range of procedures which aim to detect the presence or absence of a particular gene or chromosome mutation in an individual. It is most commonly used in the detection of genetic diseases such as sickle cell anaemia, cystic fibrosis, haemophilia, thalassaemia, and Duchenne muscular dystrophy, each of which are caused by a single gene mutation at one locus. In combination with techniques for amplification of DNA (PCR) modern screening techniques can use DNA from just a single cell to screen for several different genetic diseases. This may be done using DNA from adults (carrier detection), from a developing foetus in utero (prenatal screening), or from a fertilised ovum prior to implantation in IVF (pre-implantation screening).

Genetic screening involves a number of stages:

- ▼ Extraction of the DNA from cells (e.g. white blood cells, cheek cells, gametes, the embryonic tissues of the placenta, or undifferentiated cells in the embryo)
- ▼ Amplification of the DNA using polymerase chain reaction (PCR). This allows many copies of the required section of DNA to be produced from a single DNA molecule.
- ▼ Use of specific restriction enzymes to cut the DNA at sites within and around known gene loci.
- ▼ Use of gel electrophoresis to split the DNA fragments up on the basis of their size.
- ▼ Southern blotting and use of radioactive DNA probe to locate the fragments of DNA.
- ▼ Autoradiography to create an image of the DNA pattern for interpretation

Restriction enzymes

Restriction enzymes or restriction endonucleases 'cut' DNA at specific base sequences. Such enzymes will 'recognise' particular DNA sequences known as recognition sites, attach to these, and 'cut' the DNA. The process of cutting the DNA is known as **cleavage**, and the fragments of differing lengths produced in this way as **restriction fragment length polymorphisms** (RFLPs).

The natural function of bacterial restriction enzymes is to cut out sections of viral DNA which have entered the bacterial DNA, but in genetic screening and other genetic techniques they are used to cut a length of DNA into smaller pieces. There are several thousand types of restriction enzymes now in use whose names reflect the bacterial species from which they were extracted: thus EcoRI is derived from *E.Coli* and HindI from *Haemophilis influenzae*. Each restriction enzyme is specific to one base sequence and will digest or 'cleave' the nucleic acid (DNA) whenever such a site is encountered but at no other point. Examples of some restriction enzymes and their sites are shown below. It should be noted that some cut straight through both strands of DNA creating 'blunt ends', whilst others produce a staggered cut or 'sticky end' which is more useful in genetic engineering.

The significance of restriction enzymes in genetic screening lies in the fact that many restriction sites are located within or close to genes of interest. This means that mutations of these genes, which alter the DNA sequence, may destroy or add restriction sites. Under these circumstances DNA from individuals with the mutant gene will be cut into different length RFLPs than DNA from normal individuals when incubated with the same restriction enzyme. These length differences allow the detection of mutants using electrophoresis discussed below.

Enzyme	Source Organism	Restriction Site
<i>EcoRI</i>	<i>Escherichia coli</i>	↓ -G-A-A-T-T-C- -C-T-T-A-A-G- ↑
<i>EcoRII</i>	<i>E. coli</i>	↓ -G-C-C-T-G-G-C- -C-G-G-A-C-C-G- ↑
<i>Hae III</i>	<i>H. aegyptius</i>	↓ -G-G-C-C- -C-C-G-G- ↑
<i>HpaII</i>	<i>H. parainfluenzae</i>	↓ -C-C-G-G- -G-G-C-C- ↑
<i>SmaI</i>	<i>Serratia marcescens</i>	↓ -C-C-C-G-G-G- -G-G-G-C-C-C- ↑

Electrophoresis

This is a technique used in the analysis of DNA which has been cut into fragments of differing sizes by restriction enzymes. It is based on the principle that smaller fragments of DNA will migrate through a gel faster than larger ones when a direct electric current is applied across the gel (which is in a buffer solution). The reason for this is that the larger fragments have more difficulty moving through the dense gel. All fragments will however move as they are negatively charged and move to the positive terminal.

To perform electrophoresis a gel is made from agarose (a polysaccharide) and placed in a tank of buffer solution. DNA samples that have been treated with restriction enzymes are loaded into wells in the gel along with a dye solution. This ensures that all have equal electrical charge and that their position can be marked. A direct current is passed through the buffer solution causing the DNA to move through the gel, marked by the dye. Once the dye front has migrated about three quarters of the way down the gel the current is switched off. The position of DNA fragments on the gel cannot be seen at this stage: further steps are required for this. These are described below.

Southern Blotting

The first stage in allowing visualisation of the DNA fragments is known as Southern Blotting. There are two basic stages involved: firstly the DNA on the gel needs to be heated to cause the two strands of the helix to unwind making single stranded DNA (this is crucial as if the DNA is not single stranded it will not bind to the DNA probe);

secondly this single stranded DNA is transferred from the gel to a nitrocellulose filter or a nylon membrane. The membrane will bear an exact copy of the gel in terms of the positions of DNA fragments.

DNA probes

A DNA or gene probe is a single stranded DNA sequence usually 12 nucleotides or more in length which is complementary to a stretch of known DNA (e.g. part of a disease gene) on a chromosome. They are usually radioactively labelled using ^{32}P (the radioactive P is incorporated into the nucleotides which are used to make the probe). The probe is added to the nitrocellulose filter/nylon membrane (produced via Southern blotting) where it will attach to DNA of a complementary sequence. This is known as **hybridisation**. For example, if a particular gene contained the sequence: C C C T T C T T T C C C A A T A T then a gene probe would be made as follows.

DNA SEQUENCE: C C C T T C T T T C C C A A T A T

DNA PROBE G G G A A G A A A G G G T T A T A

The probe will bind to any region of DNA with this sequence as the nucleotides of the probe pair up with their complementary bases on the DNA. Once this has occurred excess probe is washed off the filter.

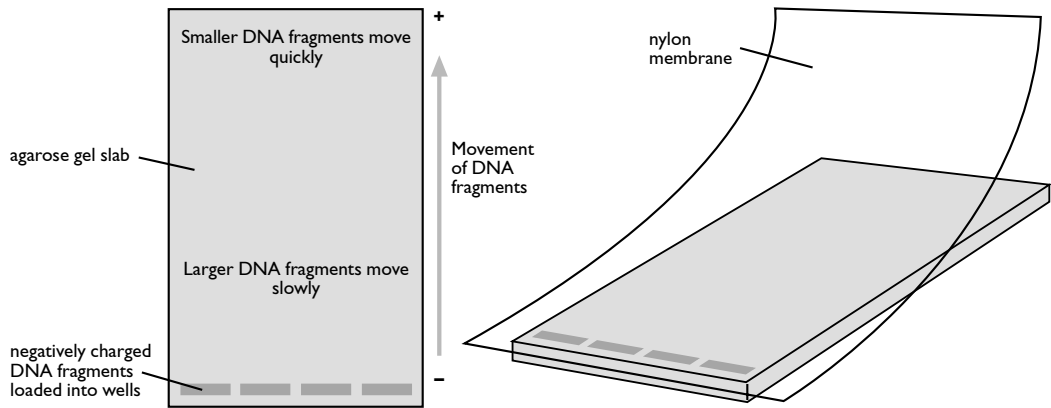
Autoradiography

In order to see the bound DNA probe and hence detect the position of DNA fragments separated in the original gel, an X-ray film is placed over the nitrocellulose filter. At sites where the gene probe has bound to DNA fragments the radioactive ^{32}P will create a black band on the film.

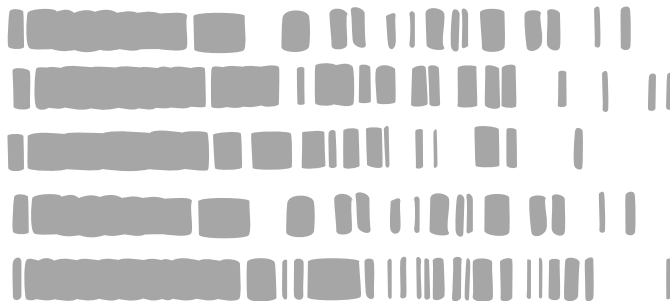
Analysis works as follows: a black band at the bottom end of the picture will correspond to small DNA fragments to which the probe has bound. Conversely, black bands at the top of the picture reveal large fragments to which the probe has bound. If a mutant gene is missing a restriction site which is present in a normal gene, then the mutant DNA will have travelled a shorter distance than the normal DNA. The positions of the bands can be compared to known 'control' DNA patterns or to the movement of DNA markers.

Stages in genetic fingerprinting

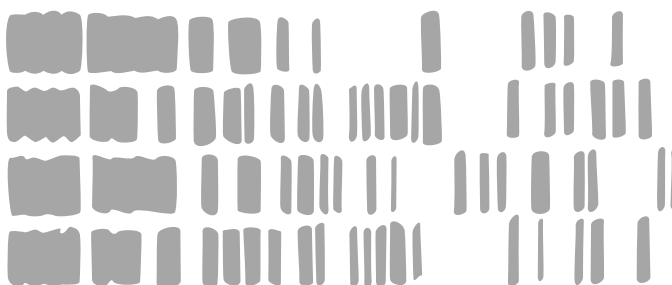
- 1 Extracted DNA e.g. from white blood cells, semen etc.
- 2 DNA cut into pieces by **Restriction Enzymes**
- 3 DNA pieces are separated by **Gel Electrophoresis** this takes 24 hours



- 4 **Southern Blotting**, the DNA fragments are transferred (blotted) onto a nylon membrane laid on top of the gel slab.
- 5 **Gene Probe Treatment** - the nylon membrane is put into a tank of single strand DNA's (probes) which are complementary to specific core DNA sequences and bind to them. The probes may be radioactive or fluorescent. Excess probes are washed off
- 6 X Ray film is placed on the membrane and the typical bands appear where the presence of **radioactive/fluorescent probes** fog the film.
- 7 The X Ray film is processed to form the **DNA Fingerprint** with its characteristic 'barcode' pattern of dark bands.

Genetic Fingerprinting - Autoradiograph bands**Bloodstain from scene of a crime****A****B****C****D**

Was individual A,B,C or D more likely to have been present at the scene of the crime?

**Mother****Child****Male 1****Male 2**

Which male is the child's father?

Enzymes and disease diagnosis

Diseases can result in changes in the concentration and distribution of enzymes in the body. Therefore the measurement of enzyme levels (enzyme assays) in body fluids can provide clinicians with valuable information about a range of diseases. This technique may be used to diagnose some of the inherited metabolic disorders where lack of a particular enzyme is the cause of the disease, e.g. very low levels of the enzyme glucose-6-phosphate dehydrogenase (G-6-P-D) in liver cells. Enzyme assays may also be used to detect changes in enzyme levels which reflect a disease process in the body, e.g. pancreatic disease (measurement of amylase, lipase and trypsin), hepatic (liver) disease (measurement of cholinesterase, alkaline phosphatase, and transaminases) and coronary heart disease (measurement of creatinine kinase). Levels of enzymes measured (usually in serum) are compared to known reference ranges for healthy individuals. In most cases such enzyme assays are used in conjunction with other investigations.

Pancreatitis

The pancreas secretes various digestive enzymes, proteases, lipases and amylases in an alkaline fluid. Some of these enzymes, notably the proteases are secreted in an inactive form and only activated in the duodenum.

Acute pancreatitis is a disease of the pancreas caused by premature activation of the protease enzymes which leads to digestion of the cells of the pancreas. The underlying causes of this are many and include gallstones, and exposure to excess alcohol. Symptoms of the disease are severe pain in the upper abdomen accompanied by nausea and vomiting. These symptoms are not very specific so doctors require results from specific tests of enzyme levels before pancreatitis can be diagnosed. Chronic (recurring) pancreatitis, usually caused by excess alcohol, can lead to permanent damage to the pancreas with implications for digestion and glucose regulation.

Acute pancreatitis can be diagnosed by measuring the levels of the pancreatic enzymes amylase and lipase in serum (blood plasma minus large proteins). Both enzyme levels are markedly increased since the cells which produce them are being destroyed allowing the enzymes to escape into the blood.

Chronic pancreatitis can be diagnosed by measuring the level of pancreatic enzymes in the intestine. Enzyme assays for the protease enzyme chymotrypsin may also be performed on faeces. In all cases the levels of pancreatic enzymes are markedly lower than in normal patients. One effect of the low lipase levels is to allow fats to pass through the intestine without being digested: this results in fat being present in the faeces.

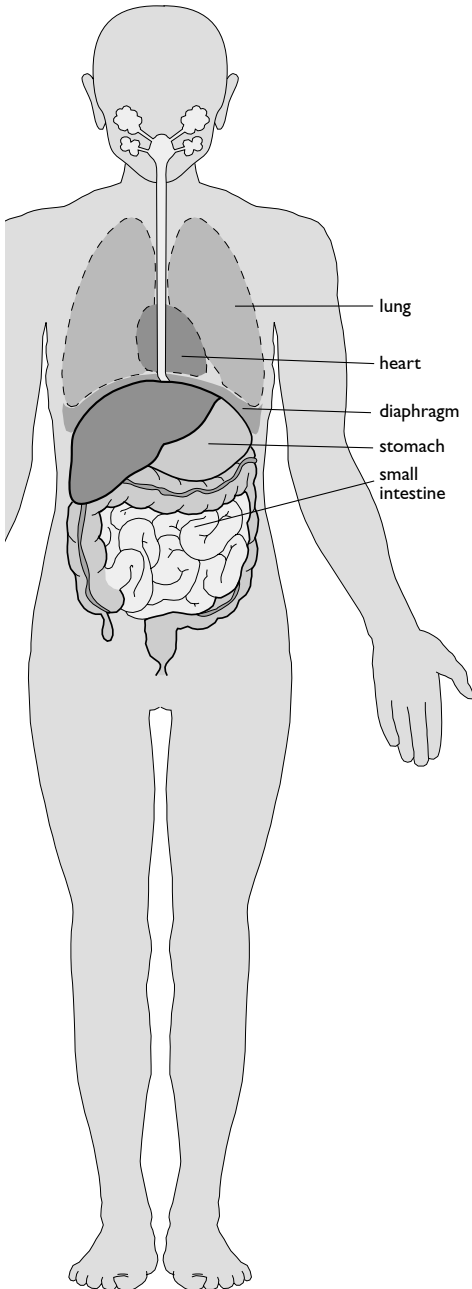
Repeat measurements of the pancreatic hormones in serum, intestine and faeces may be used to assess the course of pancreatitis and its response to treatment.

Raised levels of enzymes within the body may thus be an important diagnostic sign and a useful indicator of progression in some diseases. In other diseases the levels of biological molecules such as glucose or cholesterol are raised and can similarly be used in diagnosis or monitoring.

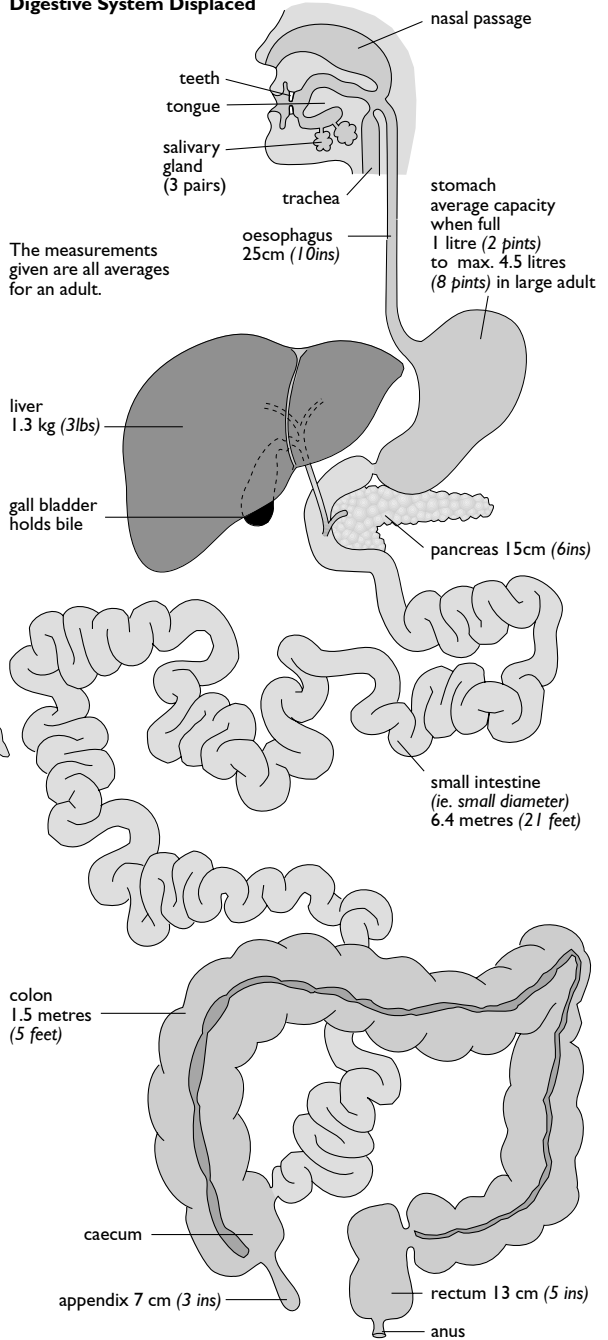
◆ CHECKPOINT SUMMARY

- ◆ A DNA probe (gene probe) is a single stranded DNA sequence usually 12 nucleotides or more in length which is complementary to a stretch of known DNA (e.g. part of a disease gene) on a chromosome.
- ◆ The use of DNA probes in diagnosis involves the following steps:
- ◆ Extraction of DNA from cells (e.g. white blood cells)
- ◆ The production of many copies of the required section of DNA (amplification) using the polymerase chain reaction (PCR)
- ◆ Use of specific restriction enzymes to cut the DNA at sites within and around known gene loci.
- ◆ Use of gel electrophoresis to split the DNA fragments up on the basis of their size.
- ◆ Southern blotting and use of radioactive DNA probe to locate the fragments of DNA.
- ◆ Autoradiography to create an image of the DNA pattern for interpretation
- ◆ Diseases can result in changes in concentration and distribution of enzymes in the body.
- ◆ The measurement of enzyme levels (enzyme assays) in body fluids can provide clinicians with valuable information about a range of diseases.
- ◆ Acute pancreatitis is a disease of the pancreas in which the protein digesting enzymes (proteases) produced by the pancreas become active within the pancreas and damage it.
- ◆ Acute pancreatitis can be diagnosed by measuring the levels of the pancreatic enzymes amylase and lipase in serum (blood plasma minus large proteins). Both enzyme levels are increased since the cells which produce them are being destroyed allowing the enzymes to escape into the blood.
- ◆ Chronic pancreatitis can be diagnosed by measuring the level of pancreatic enzymes in the intestine. In all cases the levels of pancreatic enzymes are lower than in normal patients.

Digestive System in Place



Digestive System Displaced



Enzymes as analytical reagents

Enzymes are ideal tools for use in biochemical analysis in both industry and medicine. They have revolutionised many clinical tests which aim to detect the presence of certain compounds in biological fluids (blood, plasma, urine etc) and are widely used in hospitals and laboratories. They are also used in industrial processes e.g. monitoring the production of ethanol and other materials in fermenters. Their two main advantages over other analytical reagents are:

Enzymes have a high specificity: They will only react with one specific substrate, even if that substrate is found in a complex mixture.

Enzymes have a high sensitivity: they can catalyse reactions even when the substrate is present in very low concentrations.

Enzymes are used in three main types of system

Diagnostic test strips: are plastic strips containing a pad with an immobilised enzyme(s) (see below for discussion of immobilised enzymes). These give a semi-quantitative result via a graded colour change. These are very cheap but can only be used once.

Enzyme electrodes or 'biosensors': contain an immobilised enzyme, a transducer and a tiny electric circuit. The enzyme catalysed reaction produces a product which generates an electric current in the transducer. The current is proportional to the amount of product (and hence substrate) present and is converted into a digital read out. These are small and reusable.

Enzyme analytical reactors: are large scale laboratory tools using soluble enzymes linked to dyes which test hundreds of samples per day and give results on the basis of colour changes.

Examples of enzymes used as analytical reagents in medicine and industry include :

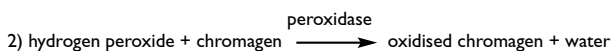
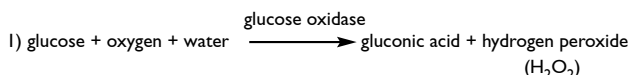
Enzyme(s)	substrate/anylate
Alcohol dehydrogenase:	alcohol
Cholesterol esterase & cholesterol oxidase	cholesterol
Glucose oxidase and peroxidase	glucose
Urease	urea
Penicillinase	penicillin

Using enzymes to test for glucose

One of the most common uses of enzymes as analytical reagents in hospitals, is in tests for blood glucose. The level of glucose in the blood is closely regulated by the pancreatic hormones insulin and glucagon. The normal level is around 90-150mg/100ml of blood. Both high and low levels of glucose are harmful and are useful indicators of disease. In particular, very high levels of glucose in the blood following meals are characteristic of the disease diabetes, as is the presence of glucose in the urine. Glucose levels in blood and urine can be detected using the two enzymes: glucose oxidase and peroxidase.

Diagnostic test strip: for testing glucose levels in urine

The colourless pad on the test strip contains the enzymes glucose oxidase and peroxidase immobilised in a cellulose mat along with a colour reagent (e.g. chromagen). When the pad is dipped into a substance containing glucose the reactions shown below occur. The end point is the blue colour (resulting from the reaction of the hydrogen peroxide with chromagen). The shade of blue reflects the concentration of glucose present and is thus semi-quantitative. The pad takes about 15 seconds to change colour.

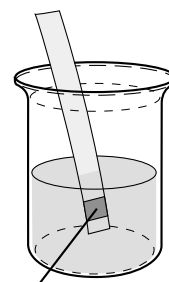


Test strips of this kind are used regularly by diabetics to check their urine for the presence of glucose. A positive result indicates that their blood glucose level was too high. Other simple test strips are available to test blood or urine for a range of compounds including urea (using urease enzyme), and cholesterol (using cholesterol esterase and cholesterol oxidase).

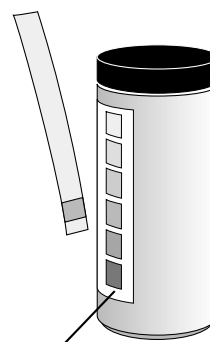
Glucose testing using a biosensor

The biosensor uses the immobilised enzyme glucose oxidase. If a substance containing glucose is placed on the test area of the biosensor the reaction noted above occurs. The gluconic acid produced by the reaction generates an electric current in the transducer which is translated into a digital readout. The read out takes less than 20 seconds to appear and gives a quantitative measure of the amount of glucose present in the sample. This method requires a very small sample: a drop of blood from a pin prick is adequate.

Biosensors, which are easy to use, relatively cheap yet also highly sensitive and specific are commonly used in hospitals to measure the levels of a range of biologically important substances in body fluids.



enzyme and chromagen impregnated pad



analysis made by comparison with colour chart on container

◆ CHECKPOINT SUMMARY

- ◆ Enzymes are used in three main types of diagnostic system
- ◆ A test strip containing glucose oxidase and peroxidase immobilised in a cellulose mat along with a colour reagent (e.g. chromagen). When the pad is dipped into a substance containing glucose a blue colour develops. The shade of blue reflects the concentration of glucose present and is thus semi-quantitative.
- ◆ Test strips of this kind are used regularly by diabetics to check their urine for the presence of glucose.
- ◆ Enzyme electrodes or 'biosensors': contain an immobilised enzyme, a transducer and a tiny electric circuit which generates an electric current proportional to the amount of substance present and is converted into a digital read out.
- ◆ Enzyme analytical reactors use soluble enzymes linked to dyes which test hundreds of samples per day and give results on the basis of colour changes.

9

Drugs are used in the control and treatment of disease

Contents

Betablockers

- ▼ Beta blockers as drugs which can be used to reduce hypertension by binding to receptor molecules.

Antibiotics

- ▼ Antibiotics used to treat bacterial disease by preventing the formation of bacterial cell walls or interfering with the processes of DNA replication and protein synthesis. The importance of the cell wall in preventing osmotic lysis.
- ▼ Bacteriostatic and bactericidal antibiotics and their effect on bacterial populations.

Monoclonal antibodies

- ▼ The use of monoclonal antibodies in enabling the targeting of specific substances and cells.

Introduction

A drug may be defined as any chemical which has an effect on the way in which the body works, although this should be expanded to include the effects of drugs on pathogens in the body. During the twentieth many new drugs have been discovered, whilst others based on naturally occurring substances e.g. in plants have been improved and developed. Successful drugs are those which treat the specific disease problem without causing unwanted side effects.

Three very different types of drug are described below. All of these drugs are specific, affecting only target cells within the body.

BETA BLOCKERS

The treatment of high blood pressure using Beta blockers

High blood pressure is one of the most common disorders in the developed world and is associated with increased risk of death from coronary heart disease, kidney disease and strokes. It refers to the pressure of the blood within arteries and has two components: systolic blood pressure, which is the pressure recorded during contraction of the ventricles and diastolic blood pressure which is the pressure recorded during relaxation of the ventricles. In a healthy young person values around 120mm/Hg over 80mm/Hg would be found. Systolic pressures over 160mm/Hg and diastolic over 95 mm/Hg could indicate high blood pressure, depending on the circumstances.

Beta blockers (or β -adrenergic antagonists) are a group of drugs used for the treatment of high blood pressure and angina (pain in the chest, coming from the heart). They work on both the heart (cardiac) muscle and the smooth muscle in the walls of arteries, and help reduce blood pressure. In particular they reduce cardiac output by slowing heart rate and reducing the force of contraction so that less blood is forced through the vessels per minute.

In the heart, Beta blockers work by binding to Beta-receptor proteins on the cell membranes of muscle cells. They therefore prevent the hormone adrenaline and neurotransmitter noradrenaline from binding to the sites and stimulating the heart to contract more strongly. They thus interfere with both nervous and hormonal stimulation of the heart, and this explains their effect in reducing systolic blood pressure.

Beta blockers also have an effect on the smooth muscle of artery walls, promoting vessel dilation (vasodilation) and hence reducing resistance of vessels to blood flow. This effect explains the reduction in diastolic blood pressure. Beta blockers also reduce anxiety which in turn reduces stress, another factor in high blood pressure.

Antibiotics and the treatment of bacterial infections

An antibiotic is a molecule produced by one microorganism which is capable of destroying or inhibiting the growth of other microorganisms. Most commonly they are chemicals produced naturally by fungi or bacteria which act on other bacteria. Antibiotics work in several ways but are often split into two main categories:

bacteriostatic antibiotics inhibit the growth and replication of bacteria;

bacteriocidal antibiotics kill other bacteria.

Bacteriostatic antibiotics do not generally do lasting damage to bacteria: if they cease to be taken then bacteria can begin to grow and divide once again.

Discovery and use of antibiotics

The discovery of antibiotics was one of the most significant events in medical history. In 1928 Alexander Fleming first noticed that the mould *Penicillium notatum* prevented the growth of bacteria on agar plates. Eleven years later, Ernst Chain and Howard Florey purified the antibiotic penicillin and tested it in mice with bacterial infections. By the mid 1940s several other antibiotics had been found and were being tested on human infections. Antibiotics were found to be extraordinarily effective at killing bacteria and allowed the first effective cures for fatal diseases like syphilis and tuberculosis. They revolutionised the treatment of a wide variety of bacterial infections and have played a major role in preventing illness and death across the world. Many infections which we consider insignificant today, such as streptococcal throat infections and the infections of wounds, caused rapid death before antibiotics were discovered.

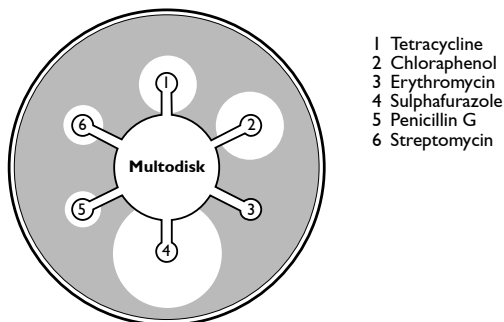
How antibiotics work

Antibiotics work by interfering with one or more of the essential functions of bacterial cells. They do not harm human cells, however, due to the differences in cell structure between bacterial (prokaryotic) and animal (eukaryotic) cells. Antibiotics are usually specific to certain types of bacteria, and thus it is common practice to find out which bacterium is responsible for an infection before attempting to treat it. Broad spectrum antibiotics will work on all or most types of bacteria but can be harmful as they also kill useful bacteria in the gut.

Antibiotics have their effects on bacterial cells in the following ways.

- ▼ Interfering with production of the bacterial cell wall.
- ▼ Limiting or preventing protein synthesis, especially the synthesis of enzymes.
- ▼ Limiting or preventing nucleic acid synthesis and hence cell division.
- ▼ Damaging bacterial cell membranes

Diagram to show action of specific antibiotics on a bacterial culture



Of these modes of action, the prevention of cell wall formation, is probably the most significant. The cell wall of bacteria is made up of long linear carbohydrate polymers called peptidoglycans which have short peptide chains cross-linking them to form a wall with high tensile strength. Antibiotics, including penicillin inhibit the synthesis and formation of these cross links. The result is a weak cell wall which will not protect the bacterium against bursting if it is in a solution that is more dilute (hypotonic) relative to the cell contents. Normally the cell wall resists further movement of water into a turgid cell, but if the wall is defective, water will continue to move into the cell by osmosis until the outward pressure on the wall is so great that it bursts (osmotic lysis).

Antibiotic resistance

When antibiotics were first used they were extremely effective at killing bacteria and hence controlling infections. Over the past fifty years, however, the excessive use of antibiotics has led to some bacteria gaining resistance to the drugs. This means that the drugs are less effective or in some cases completely useless at killing the bacteria. Resistance in the bacteria comes about through natural selection operating on chance genetic mutations in the bacteria. The short generation time and massive reproductive potential of bacteria ensures the rapid proliferation of successful mutant strains.

Two important examples of resistant bacteria (sometimes referred to as 'superbugs') are *Staphylococcus aureus* which is a common cause of infection in hospitals and some types of *Mycobacterium tuberculosis* which causes TB. Rates of illness and death from both of these bacteria are increasing. Health professionals are worried that as more bacteria become resistant to antibiotics, common infections will once again become major causes of death across the world. They are calling for a reduction in the use of the drugs both for treating mild infections, as well as in the farming industry where they are often included in animal feeds.

Monoclonal antibodies

Monoclonal antibodies are antibodies grown in the laboratory from a single B-lymphocyte clone. All the antibodies produced from such a clone are identical and will thus be specific to one antigen. As described previously (section 12.3) they are produced from cells known as hybridomas which are made by fusing a cancer cell (myeloma) with a B-lymphocyte of the desired type. Monoclonal antibodies can theoretically be produced against any antigen, not just against those on the surface of pathogens. Thus whilst they have many potential uses in the control and treatment of disease, they have a wide range of other applications, e.g. in the pregnancy testing kit where monoclonal antibodies detect the presence of the hormone HCG in the urine of a pregnant woman.

Uses of MONOCLONAL ANTIBODIES in the TARGETING of SPECIFIC SUBSTANCES and CELLS

The targeting of specific substances.

A number of research trials have used monoclonal antibodies to bind to and inactivate cytokines in the body. For example, the cytokine TNF has been implicated in a number of inflammatory disorders and infectious diseases. Anti-TNF monoclonal antibodies have been given to patients suffering from diseases such as rheumatoid arthritis, malaria, and septic shock resulting from bacterial infections. New types of monoclonal antibody are being investigated for treating overdoses with common drugs such as aspirin and paracetamol.

The targeting of specific cells

Diagnosis of cancer. Research has demonstrated the possibility of using monoclonal antibodies to detect the presence of cancer cells whose surface antigens differ from those of normal human cells. Monoclonal antibodies with fluorescent or radioactive tags could be injected into the body and used to detect cancer cells. Screening could then locate the position of the cancer cells by detecting the presence of the marker tag. Cancers could therefore be detected earlier improving the chances of treatment and recovery.

Treatment of cancer. This involves attaching toxic drugs or radioactive isotopes to monoclonal antibodies specific to cancer cells. The antibodies could then attach to the target cells destroy them, leaving non-cancerous cells unharmed.

Diagram showing mechanics of a 'magic bullet'

